UNIT-III

PLANT TISSUE CULTURE

Points to be covered in this topic

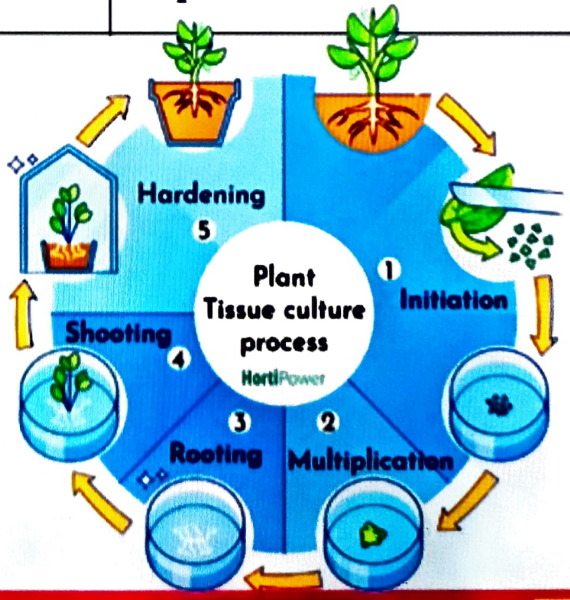
- ❑ HISTORICAL DEVELOPMENT
- ❑ TYPES OF CULTURES
- ❑ NUTRITIONAL REQUIREMENTS
- ❑ GROWTH AND MAINTENANCE
- ❑ APPLICATIONS
- ❑ EDIBLE VACCINES

PLANT TISSUE CULTURE

- Plant tissue culture is an **in-vitro technique** in which clone of plants are produced by using **plant cells, tissue of organ** under suitable environment condition or in nutrient culture.

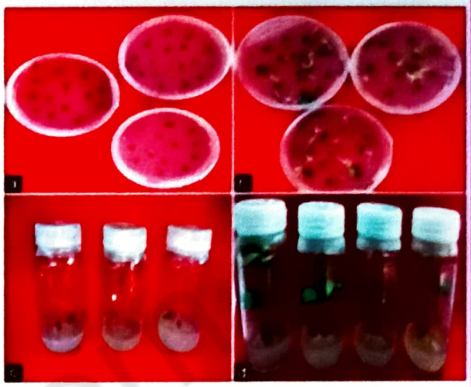
❑ HISTORICAL DEVELOPMENT

YEAR	SCIENTIST	DEVELOPMENT
1902	TLABERLAUDT	1st proposed the concept of DTC (plant tissue culture).
1904	TLANNING	Establishment of embryo culture for 1st time
1409	KUSTER	1st observation of fusion cell
1922	ROBIUS,KOTTE	In-vivo cultivation of root tips
1934	WHITE	permanent root culture for 1st time (tomato)
1934	GAVTHERET	1st permanent callus culture using vit B & auxins
1942	GAVTHERET	Observation of secondary metabolites in PTC
1953	Muir	Develop single cell culture
1955	Mothes & Kala	1st report of 2^0 metabolites production in Liquid medium.



❑ TYPE OF CULTURE

The plant growth and development occur in two different ways namely determinate growth where plant growth occurs with certain **shape and size like leaves, fruits, flowers etc.,** and indeterminate growth where plant growth occurs in the roots and stem part which is proliferate continuously.



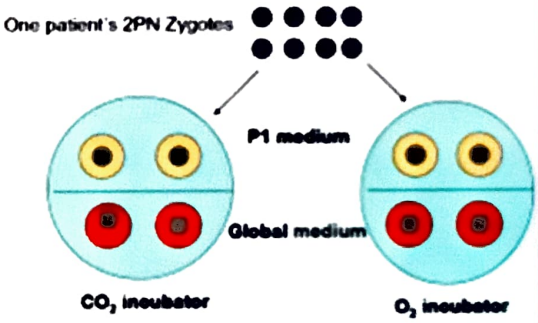
❖ **Seed culture:** Seeds are cultured in-vitro to **generate seedlings** or plants in **aseptic condition** for raising the sterile seedling. The seed culture is done to get the different kinds of explants from aseptically grown plants that help in better **maintenance of aseptic tissue**

➢ Importance:

- It is possible to independent on asymbiotic germination.
- It is important in production of Orchids.
- Increasing efficiency of germination of seeds.

❖ **Embryo Culture:** Embryo culture is a technique for cultivating an **embryo under aseptic conditions on a nutrient medium.** The method can be divided into two applications. One is performed with **mature embryos** and helps mainly in shortening the period of germination by overcoming seed dormancy.

Embryo Culture Method



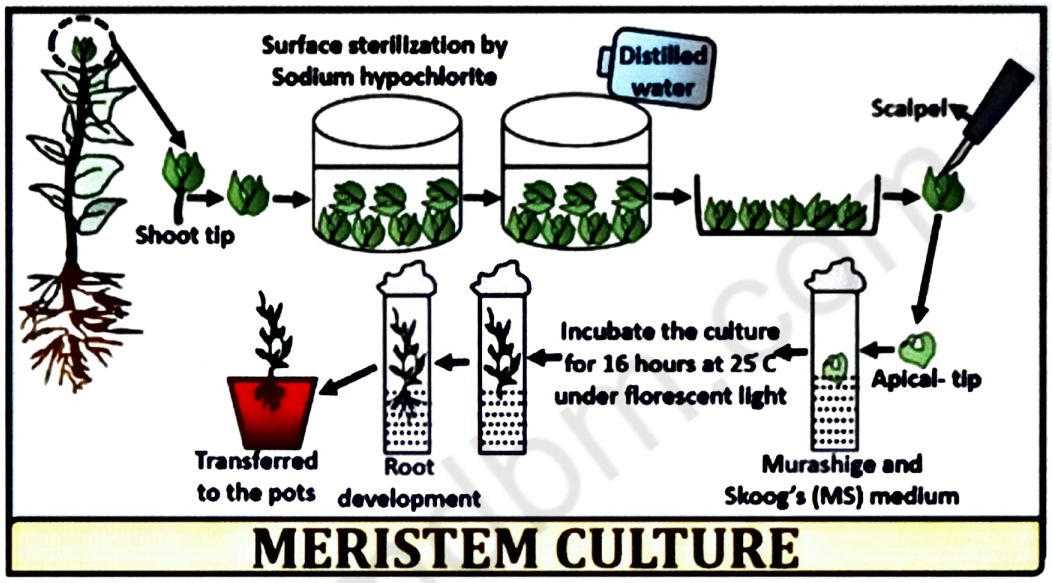
➢ Importance:

- It is useful in production of haploids.
- It helps in prevention of seed dormancy.
- It helps in shortening of breeding cycle.
- It helps in prevention of embryo abortion with early ripening stone fruits.

❖ Meristem Culture

In-vitro meristem culture

- It is the culture technique by which the apical meristem of shoots of angiosperms and gymnosperms are cultured to get the disease free plants.
- Generally meristem tips, between **0.2-0.5 mm**, most frequently produce virus-free plants and this method is referred to as meristem-tip culture.



➢ Importance:

- The method is successful in case of herbaceous plants than **woody plants.**
- It helps in culture of **Potato, Banana, Cardamom, Sugar cane, sweet potato etc.**
- It helps in production of virus free plants.
- It helps in Germplasm conservation.
- It helps in production of transgenic plants.

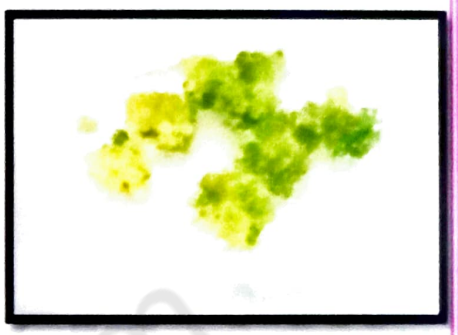
❖ Bud culture:

- Bud culture is separated into **single node culture** (stem node is used) and **axillary bud method** (where axillary buds are separated from the leaf axils and placed in high cytokinin concentration).

Importance:

- Easy step for micropropagation.
- Easy method for production of disease free plants.
- Isolation of phytoconstituents is easy

❖ **Callus culture:** It is culture of undifferentiated mass of parenchyma cell produced from an explant of a seedling or other plant part in agar medium under aseptic condition is known as Callus culture. Callus is densely aggregated, uncontrolled, undifferentiated, unorganized, aerated homogenous parenchymatous mass



Importance:

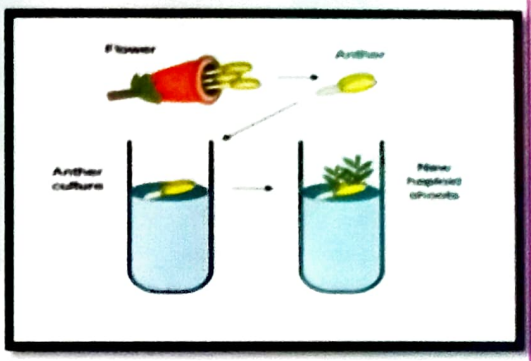
- Chromosomal variation occurs genetically or epigenetically in the cells of callus tissue.
- Increased amount of secondary metabolites are obtained by extraction of the particular callus tissue.
- This method is the source of Tissue for Cell Suspension Culture.
- Several biochemical assays are performed from callus culture

❖ **Cell Suspension Culture:** A cell suspension or suspension culture is a type of cell culture in which single cells or small aggregates of cells are allowed to function and multiply in an agitated growth medium, thus forming a suspension.

Importance:

- This culture is capable of contributing significant information about cell physiology, biochemistry, metabolic events etc.
- It is important for plant biotransformation and plant genetic engineering.
- No toxic products are formed with this culture technique.
- It helps for induction in somatic embryos and shoots.

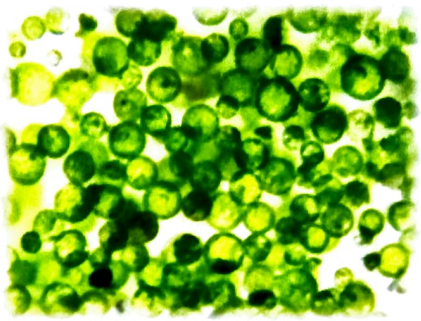
❖ **Anther Culture:** It is the in-vitro culture technique of **anther containing microspores from unopened flower bud** or immature pollen grains (Pollen culture) on a suitable nutrient medium under aseptic condition for the purpose of development of haploid plantlets.



➢ **Importance:**

- It is used for **mutation studies.**
- It is used for formation of double haploid that are **homozygous and fertile.**
- It is used to study **genetic recombination** in higher plants.
- It is used to study mode of differentiation from single cell to whole organisms.

❖ **Protoplast Culture:** They are isolated either by **enzymatic method or by mechanical method**. Mechanical method is manual method and there is a possibility of loss of protoplast cell due to friction force in motor and pestle.

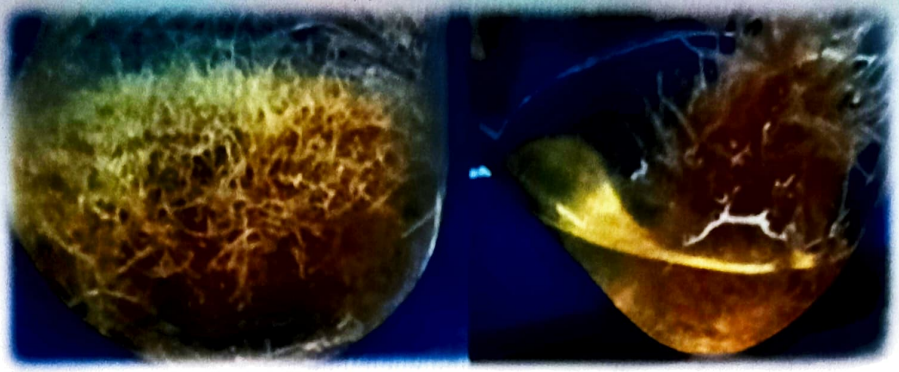


➢ **Importance:**

- This technique is used to study **Morphogenesis**.
- This technique is used to study **Photosynthesis**.
- **Protoplast cells** also can regenerate into whole plants.
- It develops novel hybrid plants through **protoplast fusion.**

❖ **Hairy Root Culture:** It is a culture produced after the infection of explants or cultures by unnaturally occurring **soil bacterium Agrobacterium rhizogenes** that contains root-inducing plasmids (Ri plasmids) are infect plant roots and cause them to produce an opines (food source for the bacterium) and abnormally very fast growth.

- Hairy roots are induced in most of the dicot plants by genetic transformation (through T-DNA, transfer DNA) with A. rhizogenes.

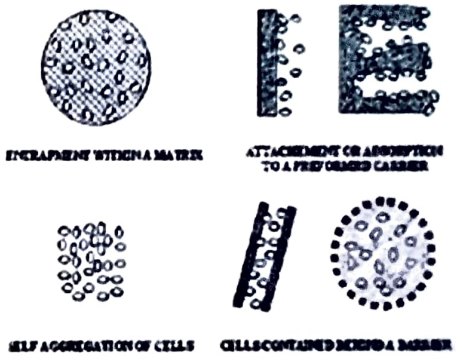


➢ Importance:

- It helps in production of high secondary metabolites.
- The culture grows under Phyto-hormone free conditions.
- The culture shows fast growth that reduces culture time and easy handling.
- It helps in functional analysis of gene.
- It also used for regeneration of whole plants.

❖ Immobilized Cell Culture:

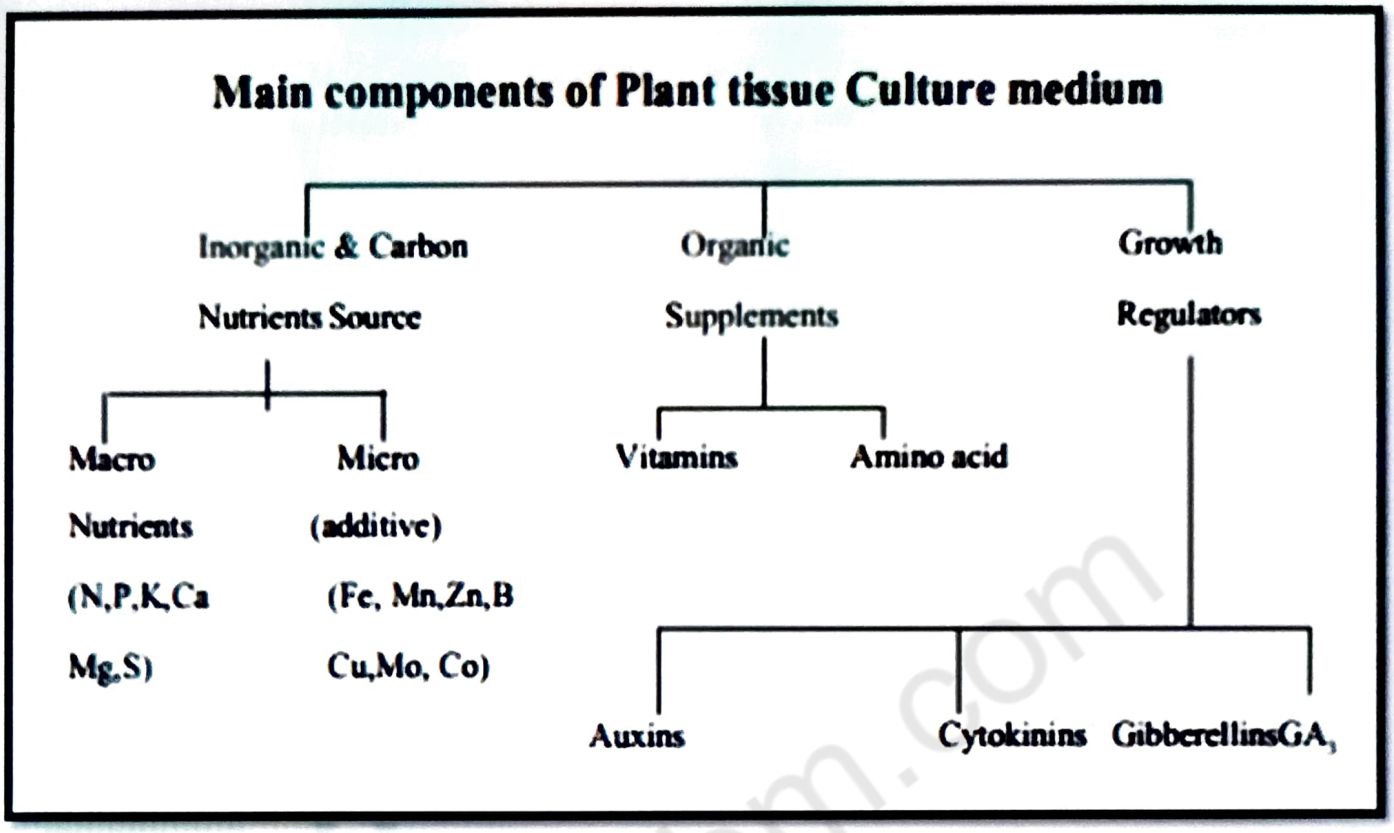
Immobilized cell technology is a method of air filtration and purification that uses whole cell immobilization. It is a process where by microfine particulate matter is removed from the air by attracting charged particulates in the air to a bio-reactive mass, or bioreactor, which enzymatically renders them inert.



➢ Importance:

- It helps in biotransformation
- Encapsulation method protects cells from mechanical damage in large fermenters.
- It is used in synthetic seed technology.
- It is used for transfer of protoplast.
- It is cultured as single cell for longer period.

❑ NUTRITIONAL REQUIREMENTS



❖ **Inorganic nutrients :** Mineral elements play very important role in the growth of plant Function of nutrients in plant growth. Essentially about **15 elements found important for whole plant growth** have also been proved necessary for the growth of tissue(s) in culture.

➢ **Macronutrient :** Elements required in the life of a plant greater than 0.5 mmol/lit are referred as macronutrients.

The macronutrients include six major elements as follows:

Nitrogen (N), Potassium (K), Phosphorous (P). Calcium (Ca). Magnesium (Mg). Sulfur (S).

➢ **Micronutrient :** like **boran, copper, iron, manganese, zinc etc.**

❖ **Organic nutrients :** Includes Vitamins like **Vitamin B_1, B_6, B_3, B_5, etc. Amino acids like L-arginine, L-asparagine. L-cysteine HCL, L-glutamine etc, Carbon source like glucose or maltose.**

❖ **Growth regulators** - Plant hormones play an important role in growth and differentiation of cultured cells and tissues. **Involves namely**: Auxins, Cytokinins, Gibberellins, Abscisic acid, and Ethylene.

1. **Auxin-Roots.**
2. **Cytokinin-Shoots**
3. **Gibberellin – Cell**

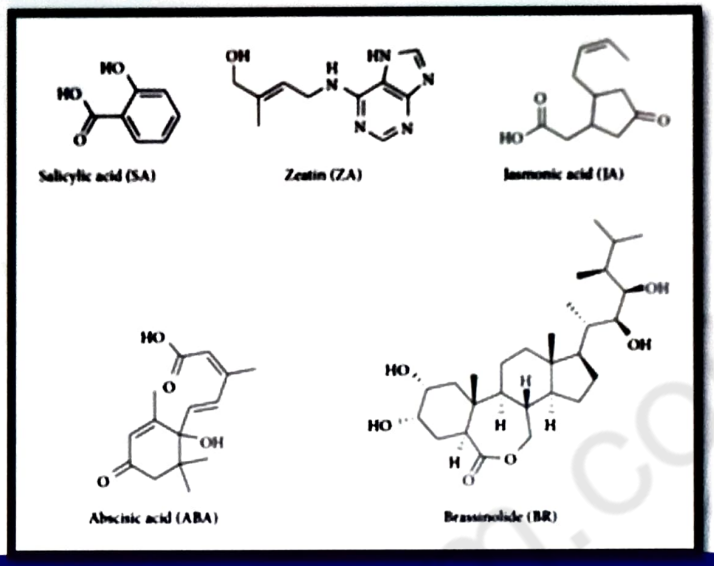


Fig. - Structures of various growth regulators

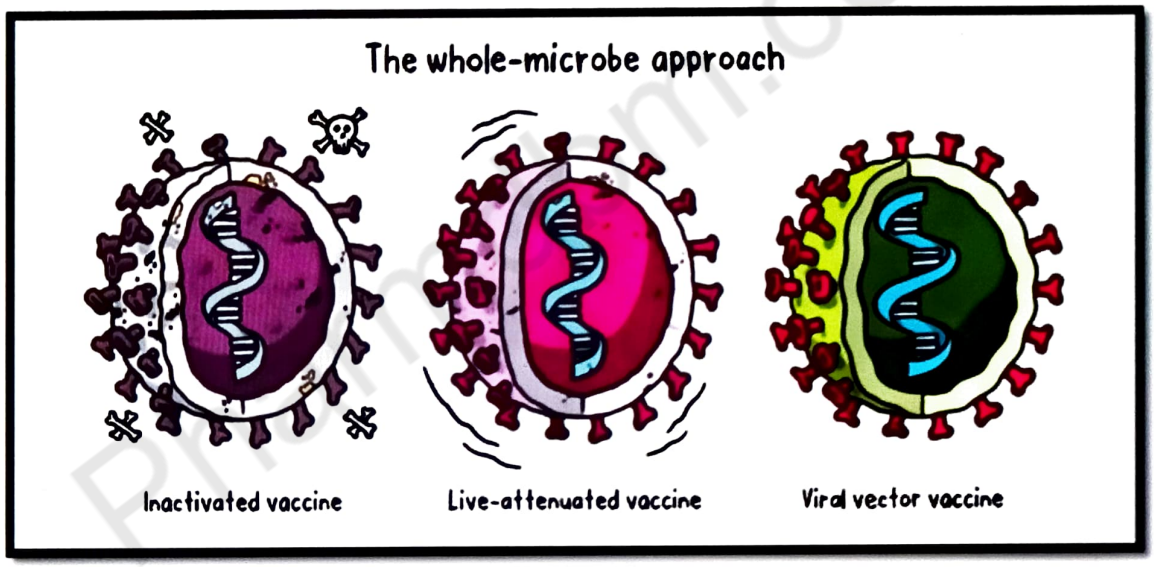
❖ **Others media substances :** like **protein hydrolysates, yeast extracts, fruit (e.g. banana) extracts, coconut milk, solidifying agents like agar, alginate, gelatin etc., Iron source e.g. EDTA. Antibiotics.**

❑ APPLICATIONS OF PLANT TISSUE CULTURE IN PHARMACOGNOSY

- **Production of biomass energy** which is useful in forestry.
- **Somatic Embryogenesis and Synthetic Seed** development by which the mass production of adventitious embryos occurs that ultimately develop into complete plantlet in maturing media.
- **Propagation of haploid Plants** through anther or pollen culture (androgenesis) or through ovaries or ovule culture (gynogenesis).
- **Development of Transgenic Plants** by carrying genes for different traits like insect resistance, herbicide tolerance, delayed ripening, increased amino acid and vitamin content, improved oil quality, etc.

❑ EDIBLE VACCINES

- A vaccine is a biological preparation that provides **active acquired immunity to a particular disease**. Vaccine consists of dead pathogens or live but **attenuated organisms.**
- There are three types of traditional vaccines namely, **inactivated or killed vaccines** (examples: Typhoid, Cholera, Plague, Rabies etc.), **live attenuated vaccines** (examples: BCG, Typhoid oral, Oral polio, Measles, Mumps etc.) and **toxoids** (examples: Diphtheria, tetanus).
- They induce immunity against pathogen either by production of antibodies or by activation of T-lymphocytes.
- These vaccines are **easily administrable, storable** and widely acceptable as bio-friendly in all developing countries.



➢ Examples:

- Plants used for edible vaccines are **like Tobacco, Potato, Banana, Tomato, Rice, Carrot, Corn, Muskmelon, Soybean etc.**
- It has a major drawback that it needs to be eaten as raw because cooking causes denaturation of protein and makes it ineffective.
- The first successful human trial for an edible vaccine was conducted in **year 1997** in which volunteers were fed transgenic potatoes, which possessed the b-subunit of the E. coli heat-labile toxin, responsible for diarrhoea.

Preparation:

- **Live attenuated vaccines** are prepared by grown disease causing organism under **special laboratory conditions** that causes loss of virulence or disease causing properties.
- The attenuation is obtained by **heat** or **by passage of the virus** in foreign host like tissue **culture cells or embryonated eggs.**
- Cell culture is required for the viral vaccines because viruses are replicated inside the living cells.
- **Example:** Sabin polio vaccine is produced by attenuated with high inocula and rapid passage in primary monkey kidney cells. Inactivated vaccines are produced by killing the disease causing microorganism with heat or by chemicals. Vaccines are also produced by gene techniques where single gene is expressed in a foreign host by cloning.

Advantages of Edible Vaccines:

- They are safe as they do not contain **heat-killed pathogens.**
- Edible vaccines have **efficient mode of action for immunization.**
- They do not need **sophisticated equipments and machines** as they are easily grown on rich soils
- They are widely accepted as they are orally administered unlike traditional vaccines that are injectable.
- They are **comparatively cost effective**, as they do not require cold chain storage.

Importance:

- It is used for **cancer therapies** like colon cancer and cervical cancer.
- It is applied for **many infectious diseases** like AIDS, tetanus, small pox, measles, plague, foot and mouth disease, tuberculosis, influenza etc.
- It is used for **autoimmune diseases** like Type-I diabetes and multiple sclerosis.