TISSUE CULTURE

- It is a laboratory technique which involves growing cells or tissues or organs on artificial nutrient media.
- It is also known as *in vitro* culture or **Micropropagation.**
- It is based on the principle of **Cellular totipotency**.
- Inherent capacity of cell to divide and differentiate into an organism is called as cellular totipotency.
- Usually plant cells are totipotent but not animal cells.
- The various steps of plant tissue culture are
  1. Preparation of Nutrient culture medium
  2. Sterilisation of Culture medium
  3. Preparation of Explant
  4. Inoculation of Explant
  5. Incubation for growth
  6. Acclimatization of plantlets and transfer to pots.

1. Preparation of Nutrient Culture medium

- A plant obtains water and minerals from soil, gases from atmosphere and energy from sun. All these have to be provided in the laboratory for plant tissue culture.
- A nutrient medium consists of all essential micronutrients, macronutrients, carbohydrates, vitamins and aminoacids.
- The above nutrients are dissolved in distilled water and its pH is adjusted to **5.6 to 6.0**.
- Plant parts are provided supported by solidifying the nutrient medium by adding **Agar-Agar**.
- The most commonly used culture medium in tissue culture is **Murashige** and **Skoog** (M.S.) medium.
- Nutrient medium without any growth regulators is called as **Minimal medium** or **basal medium**. It is used for seed germination to obtain aseptic seedlings and in callus cultures.
- To obtain complete plants from callus the nutrient medium is supplemented with growth regulators such as Auxins (**IAA, NAA, 2-4 D**), Gibberellins, Cytokinins (**Kinetin** and **Benzyl Adenine**) and several other compounds.
- The culture medium is dispensed into culture vials and the mouths are plugged with **non – absorbent** cotton to promote exchange of gases and prevent entry of microorganisms.

2. Sterilization of Culture Medium

- The above prepared culture medium provides luxuriant growth of various unwanted microorganisms that check the growth of plant tissue. Hence it has to be made microbial free.
- For the purpose the medium is sterilised by autoclaving at **121°C** and **15 lb** pressure for **15** minutes.
- They are examined for microbial growth the next day. Only non – contaminated ones must be used for inoculation.

3. Preparation of Explant

- Any living part of the plant used for culturing in tissue culture is called as **explant**.
- The explant of a garden or potted plant carries germs on its surface. Hence its surface must be cleaned by a detergent in running water and surface sterilised with disinfectants like **Sodium hypochlorite** solution and later rinsed in distilled water.
- Aseptic explants can be obtained from aseptic seedlings developed in tissue culture from inoculated seeds. These seeds are surface sterilised with **Mercuric chloride** solution and rinsed in distilled water before inoculating in MS medium.

4. Inoculation of Explant

- The prepared explant must be incorporated into culture vials under aseptic conditions such as in laminar air flow chambers that provide aseptic conditions.

5. Incubation for growth

- The inoculated culture tubes must be subjected to incubation for **3 – 4** weeks in suitable conditions such as temparature, humidity and illumination.
- During this period the cells of explant divide and form a mass of undifferentiated cells known as **Callus**. Sometimes it produces roots and shoots directly
- Explant develops into callus when grown in medium containing only **auxin** (2-4 D).
- Production of roots and shoots from callus or explant by growing in different concentrations of auxins and cytokinins is called as **Organogenesis**.
- High concentration of auxin induces root formation (Rhizogenesis) and cytokinin induces shoot formation (**Caulogenesis**). After formation of roots, it is transferred to medium containing auxin for root formation.
- Sometimes the explant forms embryogenic callus from which somatic embryos or embryoids are formed. Embryoids can be directly formed from explant too.
- Embryoids formation pass all developmental stages as seen in embryogeny.

- Embryoids are transferred to another culture tube where they develop into plantlets or complete plants.
- The embryoids can be encapsulated by coating with **sodium alginate** to form **synthetic seed** or **artificial seeds** which can be stored and later sown.

6. Acclimatization of Plantlets and transfer to pots

- The plants obtained by above process must be acclimatised before their transfer to pots.
- The plants are gently washed to remove the traces of culture media and kept in plastic pots containing **soil – rite** (A substance prepared from Coconut shell and other organic materials).
- The pots must be covered with polythene bags and kept in the laboratory at room temparature for **1 – 2** weeks.
- Polythene bags provide high humidity to plants for acclimatization.
- The polythene bags are gently and gradually removed to ensure acclimatization.
- If the plants appear strong and healthy they have to be transferred to normal pots with **soil** and **manure**.

Organ culture

A. Anther Culture

- It is growing anthers of unopened flowers on artificial media to obtain callus and haploid plants.
- It was first carried out by **Guha** and **Maheswari** in *Datura innoxia*.
- **Nitsch** further advanced this technique by obtaining complete plants from pollen grains of **Tobacco**.
- **Obtaining haploid plants** is the main objective of Anther culture. It is based on the principle of totipotency and presence of only one set of chromosomes in pollen grains.
- *In vitro* obtaining of haploid plants from isolated pollen grains is called as **Androgenesis**.

Method:

- The excised and surface sterilised anthers are transferred to nutrient medium and the cultures are maintained at **25 – 28°C** for **3 – 4** weeks and haploid plants are obtained.
- When the plants are **50 mm** tall, they are transferred to pots containing **compost** and covered with **glass beakers** or **polythene bags** to prevent dessication.
- After **one week** they have to be transferred to larger pots. These can grow and flower but fail to set seeds due to the presence of only one set of chromosomes in their cells.
- From these haploid plants, homozygous diploids can be obtained by **colchicine** treatment.

Applications

1. **New genotypes** are produced from haploids. These are made **homozygous** diploids.
2. **Recessive mutations** are easily detected in haploid plants.
3. Haploid plants serve as good material for **biochemical** and **physiological** studies.
4. These are useful in genetic engineering. Genes of E. coli can be transferred to haploid tomato plants.
5. Production of haploid plants **shorten the breeding period** and **recovers** several gene combinations. These are valuable material for crop improvement.

B. Embryo culture

- It is culturing the zygotic embryos of different developmental stages on nutrient media.
- **Hanning** was first to grow the embryos of *Raphanus sativus.*
- **Laibach** recovered the progeny of interspecific hybrids of *Linum* by this culture.
- **Embryos** are isolated aseptically and transferred to nutrient medium.
- Young embryos can be grown well on nutrient medium containing growth regulators, vitamins and amino acids.
- Mature embryos can be grown on basal medium. Plants can be obtained in **4 – 5** weeks.

Applications

1. Aborted embryos due to underdevelopment can be recovered. This is called as **Embryo rescue**.
2. Weak embryos obtained due to crosses between incompatible parents and distant genera can be grown on nutrient media and recoverd.
3. Prolonged dormancy of certain embryos can be reduced and seedlings are obtained.
4. Metabolic and biochemical aspects of **seed dormancy** can be understood.
5. Obligate parasites like **Cuscuta** sp. can be grown without host by this culture.

Plant Tissue Culture – Applications

1. A large number of plants are produced in smaller area and short time. This is known as **Micropropagation**. It is used to propagate ornamental, exotic, plantation, fruit yielding and orchid plants.
2. Variations seen in plants obtained through tissue culture are known as **somaclonal variations**. These are exploited in crop improvement.
3. In plants like papaya only female plants are required. By tissue culture only female plants can be obtained by growing the tissues of female plant.
4. **Virus free plants** can be obtained by **shoot tip culture**.
5. **Seed dormancy** problems can be overcome and rare hybrids can be obtained.
6. **Sodium alginate** encapsulated embryoids known as synthetic seed can be stored and transported.

7. Haploid plants are obtained through anther culture and they can be made homozygous diploids by treating with colchicines.
8. High value products of plants with industrial and medicinal significance can be produced in vitro.
9. Transgenic plants production is completely dependent on plant tissue culture.
10. Plants are stored in the form of tissues and artificial seeds at low temperature. It is known as **cryopreservation** (in liquid nitrogen at –196°C). In this way tissue culture plays a role in conservation of plant biodiversity.