Day - Friday

Date 3/7/2020

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UNIT - 3rd

PLANT TISSUE CULTURE

Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition.

Plant tissue culture is widely used to produce clones of a plant in a method known as micropropagation.

Advantage of Plant Tissue culture

The production of exact copies of plants that produce particularly good flowers, fruits.

To quickly produce mature plants.

The production of multiples of plant in the absence of seeds.

The regeneration of whole plants from plants cells that have been genetically modified.

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The production of plants from seeds.

The production of plants in sterile containers that allows them to be moved with greatly reduced chances of transmitting diseases, pests and pathogens.

Advantages of Plant Tissue culture

1. Availability of raw material.
2. Variation in supplies and quality
3. Patent rights.
4. Easy purification of the compounds.
5. Modification of chemical structure.
6. Disease free and desired product.
7. Crop improvement.

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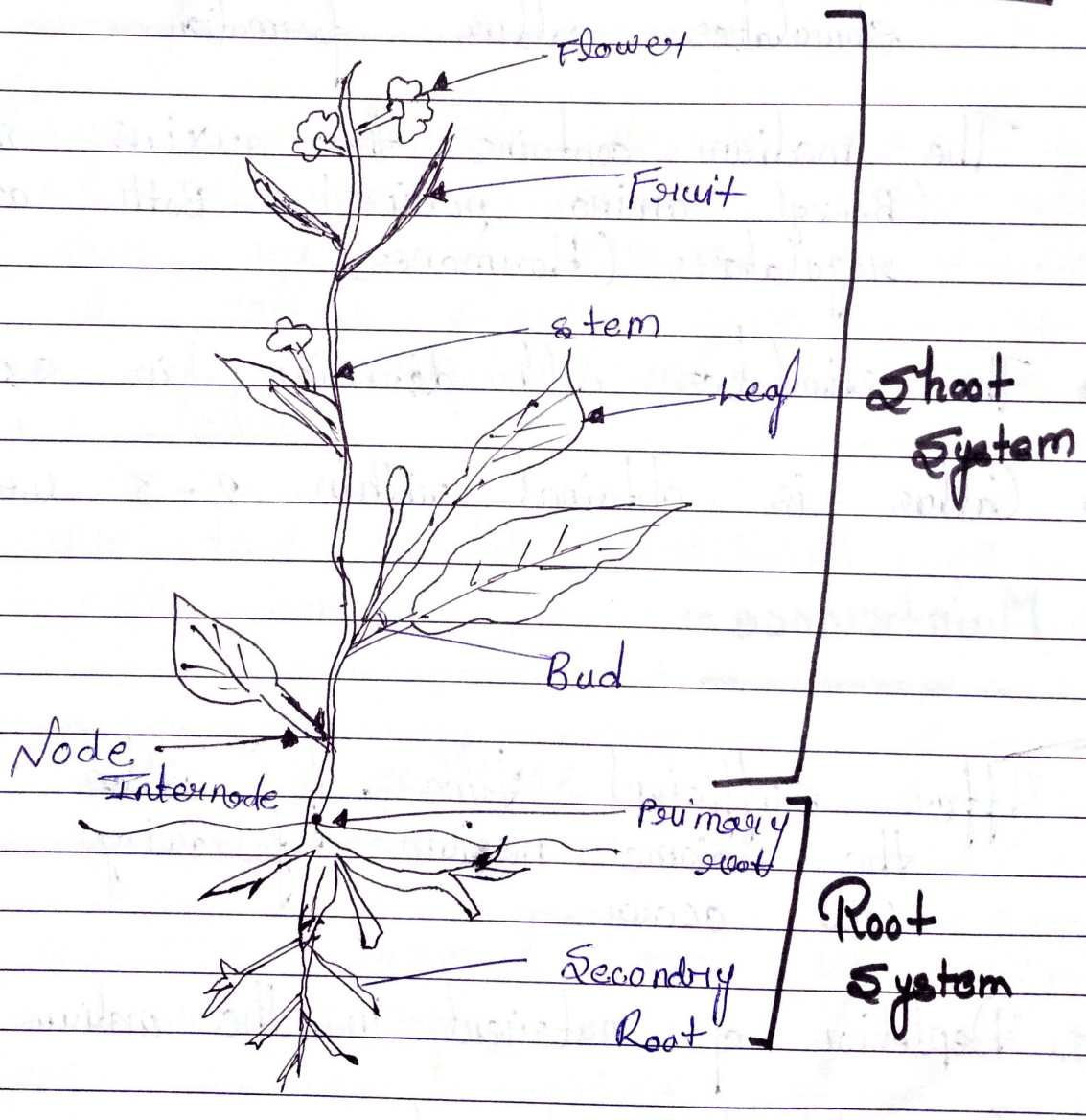
Explant

Any part of a plant taken out and grown in test tube under sterile conditions in special nutrient media is called explant.

Types of Plant Tissue culture



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[1] Callus Culture

- In Callus culture, cell division in explant forms a callus.
- Callus is irregular unorganized and undifferentiated mass of actively dividing cells.
- Darkness and solid medium gelled by agar stimulates callus formation.
- The medium contains the auxins and BAP (Benzyl amino purines). Both are growth regulators (Harmones).
- The stimulates cell division in explant.
- Callus is obtained within 2-3 weeks.

Maintenance -

After sufficient time of callus growth on the same medium following changes will occur -

1. Depletion of nutrient in the medium.
2. Gradual loss of water.

3. Accumulation of metabolic toxins.

- Hence for maintenance of growth in callus it is necessary to subculture the callus.
- Subculture should be repeated after 4-5 weeks.

[2] Single cell culture

As states earlier, cells devided from a single cell through mitosis constitute a clone and the process of obtaining clones is called cloning (asexual progency of a single individual make up a clone).

There are two popular techniques for single cell culture.

[3] Root Tip culture

Tips of the lateral root are sterilized, excised and transferred to fresh medium.

The lateral roots continue to grow and provide several roots.

After 7 days that are used to initiate stock or experiment.

Thus the root material derived from a single radical.

Such genetically uniform root cultures are ~~reff~~ referred to a clone of isolated roots.

[4] Leaves Culture

Leaves (800 um) may be detached from shoots, surface sterilized and place in healthy condition for long period.

Growth rate in the culture depends on their stages of maturity at excision.

Young leaves have more growth potential then the nearly mature ones.

[5] Shoot tip culture

The excised shoot tips (100 - 1000 µm long) of many plant species can be cultured on relatively simple nutrient media.

This media must contains growth harmones and will often from roots and develop into whole plants.

[6] Complete Flower culture

Flowers (2 days after pollination) are excised. sterilized by immersion in 5% calcium hypochloride. washed with sterilized water.

Transfer this to culture tubes containing an agar medium.

Fruits which develop are smaller than their natural counterpart. size can be increases by supplimenting the medium with appropriate combination of growth harmones.

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[7] Anther Culture

Young Flower buds are removed from the plant and surface-sterilized.

The anthers are then excised and transferred to an appropriate nutrient medium.

The plantlet are formed after 4-5 weeks of inoculation.

Many plantlets are produced from the single anther.

[8] Pollens Culture

Pollen grains are removed from the anther.

Anthers are placed in a 5ml liquid medium in petri dish.

Petri dishes containing the pollen ~~grant~~ grains in the culture media are sealed with parafilm and incubated at 28° C in dark for 14 days.

3-4 weeks may be required to obtain haploid plantlets.

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Application of Tissue Culture

- Tissue culture is used to conserve the rare species in the forest.
- A plant breeder may use tissue culture to screen cells rather than plants.
- Large-scale growth of plant cells in liquid culture as a source of secondary products, like recombinant protein used as biopharmaceuticals.
- Help in crop improvement.
- Creation of additional genetic variation.
- Selection of plants resistant to toxins, ~~to~~ viruses etc.
- Production of disease free plants.
- Selection of plants resistant to toxins, viruses etc.
- Production of many plants that are clone to each other.

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Methods of Plant Tissue Culture

It includes two major methods -

1. (A) Type of in vitro growth - Callus and Suspension cultures.

2. (B) Type of explant -

Single cell culture, shoot and root cultures, somatic embryo culture, Meristem culture, anther and haploid production, protoplast culture and somatic hybridisation, embryo culture, ovule culture, ovary culture etc.

Environmental Conditions

There are three important aspects in vitro culture -

1. Nutrient Medium
2. Aseptic condition
3. Aeration of the tissue.

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[1] Nutrient Medium

Medium depends upon the type of plant tissue or cell used for culture.

Generally nutrient consist of -

→ Inorganic Salts [Both micro and macro element]

→ A carban source [Usually sucrose]

→ Vitamins (eg - Nicotinic acid, thiamine, pyridoxine)

→ Amino acids (eg - Arginine)

→ Growth regulatores (eg - Auxins)

→ An optimum pH (5.7) is also very important

[2] Aseptic Condition

Nutrient medium contains sugar which increases growth of microbes.

These microbes compete with growing tissue and finally kill it.

It is important to maintain aseptic condition

Sterilization is very important to stop the growth of microbes.

[3] Aeration of the Tissue

Proper aeration of the cultured tissue is also an important aspect of culture technique.

It is achieved by occasionally stirring the medium by stirring or by automatic Shaker.