PLANT BIOTECHNOLOGY

(E CONTENT)

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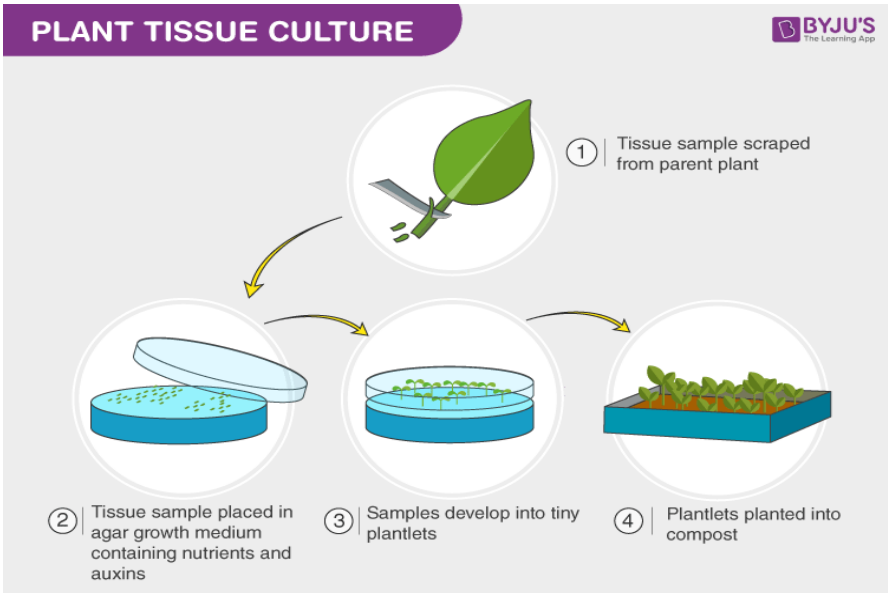
Plant tissue culture

Plant tissue culture was a new addition to the methods of plant breeding that developed around the 1950s. Since the conventional breeding techniques could not fulfil the required demand of crops, tissue culture came around as a grand leap in breeding practices. It makes use of parts of a plant to generate multiple copies of the plant in a very short duration. The technique exploits the property of totipotency (plant cells have the ability to divide and form a new organism) of plant cell which means that any cell from any part of the plant can be used to generate a whole new plant.



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. It is widely used, to produce clones of a plant in a method known as micropropagation (or tissue culture is the practice of rapidly multiplying plant stock material to produce many progeny plants, using modern plant tissue culture methods). Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation, including:

In vitro tissue culture of potato explants

Plant tissue cultures being grown at a USDA seed bank, the National Center for Genetic Resources Preservation.

- The production of exact copies of plants that produce particularly good flowers, fruits, or other desirable traits.
- To quickly produce mature plants.
- To produce a large number of plants in a reduced space.
- The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.
- The regeneration of whole plants from plant cells that have been genetically modified.
- The production of plants in sterile containers allows them to be moved with greatly reduced chances of transmitting diseases, pests, and pathogens.
- The production of plants from seeds that otherwise have very low chances of germinating and growing, i.e. orchids and Nepenthes.
- To clean particular plants of viral and other infections and to quickly multiply these plants as 'cleaned stock' for horticulture and agriculture.
- Reproduce recalcitrant plants required for land restoration
- Storage of genetic plant material to safeguard native plant species.

Plant tissue culture relies on the fact that many plant parts have the ability to regenerate into a whole plant (cells of those regenerative plant parts are called totipotent cells which can differentiate into various specialized cells). Single cells, plant cells without cell walls (protoplasts), pieces of leaves, stems or roots can often be used to generate a new plant on culture media given the required nutrients and plant hormones.

Types of Plant tissue culture

1. Seed Culture

2. Embryo Culture
3. Callus Culture
4. Organ Culture
5. Protoplast Culture
6. Anther Culture

Procedure of Plant tissue culture

The part(s) of the plant used for culturing is known as explants. The explants are cultured in-vitro on a nutrient medium that caters to fulfil its nutritional requirements. The nutrient medium must provide the following:-

1. **Macronutrients** – This includes elements like nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulfur (S) which is required for proper growth and morphogenesis.
2. **Micronutrients** – Elements like iron (Fe), manganese (Mn), zinc (Zn) etc., which are also crucial to the growth of tissues.
3. **Carbon or Energy source** – This is one of the most crucial ingredients of the nutrient media. Sucrose is the most widely used carbon source among other carbohydrates that serve to provide C, H, and O.
4. Vitamins, amino acids, and other inorganic salts.

Apart from these, the culture media also serves as a medium for supplying phytohormones or plant growth regulators to the issues which bring about their morphogenesis as per requirement. The tissues of the explants first lose their specificity to form a hard brown lump known as callus. The callus then splits to develop a plant organ or a whole new plant depending upon the quantity and composition of phytohormones supplied. The entire process requires strict aseptic conditions to be maintained at all times as a single contamination can ruin an entire batch of plants.

Uses of Plant tissue culture

Tissue culture is used to develop thousands of genetically identical plants from one single parent plant known as somaclones (plants that are produced through tissue culture), and this process is

known as micropropagation. The method offers an advantage over other methods as it can be used to develop disease free plants from disease-rode plants by using their meristems (apical and axillary) as explants.

Since this method produces new plantlets by the score of thousands, it has been used extensively for the production of commercially important plants including food plants like tomato, banana, apple etc. The most notable example of the application of micropropagation was observed in the farming of orchids as it rose exponentially due to the availability of millions of plantlets due to tissue culture methods.

Applications of plant tissue culture

Plant tissue culture is used widely in the plant sciences, forestry, and horticulture. Applications include:

- The commercial production of plants used as potting, landscape, and florist subjects, which uses meristem and shoot culture to produce large numbers of identical individuals.
- To conserve rare or endangered plant species.
- A plant breeder may use tissue culture to screen cells rather than plants for advantageous characters, e.g. herbicide resistance/tolerance.
- Large-scale growth of plant cells in liquid culture in bioreactors for production of valuable compounds, like plant-derived secondary metabolites and recombinant proteins used as biopharmaceuticals.
- To cross distantly related species by protoplast fusion and regeneration of the novel hybrid.
- To rapidly study the molecular basis for physiological, biochemical, and reproductive mechanisms in plants, for example in vitro selection for stress-tolerant plants.
- To cross-pollinate distantly related species and then tissue culture the resulting embryo which would otherwise normally die (Embryo Rescue).
- For chromosome doubling and induction of polyploidy, for example doubled haploids, tetraploids, and other forms of polyploids. This is usually achieved by the application of antimitotic agents such as colchicine or oryzalin.

- As a tissue for transformation, followed by either short-term testing of genetic constructs or regeneration of transgenic plants.
- Certain techniques such as meristem tip culture can be used to produce clean plant material from virused stock, such as sugarcane, potatoes and many species of soft fruit.
- Production of identical sterile hybrid species can be obtained.
- Large scale production of artificial seeds through somatic embryogenesis.

20. PLANT GROWTH REGULATORS

Plant growth regulators or phytohormones are organic substances produced naturally in higher plants, controlling growth or other physiological functions at a site remote from its place of production and active in minute amounts. Thimmann (1948) proposed the term *Phyto hormone* as these hormones are synthesized in plants. *Plant growth regulators* include auxins, gibberellins, cytokinins, ethylene, growth retardants and growth inhibitors. Auxins are the hormones first discovered in plants and later gibberellins and cytokinins were also discovered.

Hormone

An endogenous compound, which is synthesized at one site and transported to another site where it exerts a physiological effect in very low concentration. But ethylene (gaseous nature), exert a physiological effect only at a near a site where it is synthesized.

Classified definition of a hormone does not apply to ethylene.

Plant growth regulators

- Defined as organic compounds other than nutrients, that affects the physiological processes of growth and development in plants when applied in low concentrations.
- Defined as either natural or synthetic compounds that are applied directly to a target plant to alter its life processes or its structure to improve quality, increase yields, or facilitate harvesting.

Plant Hormone

When correctly used, is restricted to naturally occurring plant substances, there fall into five classes. Auxin, Gibberellins, Cytokinin, ABA and ethylene. Plant growth regulator includes synthetic compounds as well as naturally occurring hormones.

Plant Growth Hormone

The primary site of action of plant growth hormones at the molecular level remains unresolved.

Reasons

- Each hormone produces a great variety of physiological responses.
- Several of these responses to different hormones frequently are similar.
- The response of a plant or a plant part to plant growth regulators may vary with the variety of the plant.
- Even a single variety may respond differently depending on its age, environmental conditions and physiological state of development (especially its natural hormone content) and state of nutrition. There are always exceptions for a general rule suggesting the action of a specific growth regulator on plants.
- There are several proposed modes of action in each class of plant hormone, with substantial arguments for and against each mode.

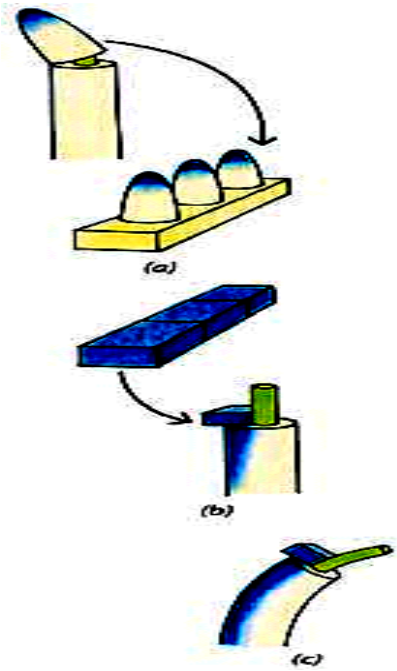
Hormone groups

Auxin	-	Substances generally resembles IAA and has the ability to stimulate the elongation of coleoptiles.
Gibberellins	-	are diterpenoids, which have the ability to elongate the stem of green seedlings especially certain dwarf and rosette types.
Cytokinin	-	Usually substituted Adenines, which resembles zeatin (Naturally occurring cytokinin in *Zea mays*) and have the ability to stimulate cytokinensis in cultures of tobacco cells.
Ethylene	-	Gaseous regulator that stimulate is diametric growth in the apices of dicot seedlings.
Inhibitors	-	are regulators of growth, which originally depress the

Auxins

Auxins are a group of phytohormones produced in the shoot and root apices and they migrate from the apex to the zone of elongation. Auxins promote the growth along the longitudinal axis of the plant and hence the name (auxeing : to grow). The term, auxin was introduced by Kogl and Haagen- Smit (1931). Went (1928) isolated auxin from the Avena coleoptile tips by a method called *Avena coleoptile or curvature test* and concluded that no growth can occur without auxin. Auxins are widely distributed through out the plant however, abundant in the growing tips such as coleoptile tip, buds, root tips and leaves. Indole Acetic Acid (IAA) is the only naturally occurring auxin in plants. The synthetic auxins include,

Avena Curvature Test



Cut off the tips of Avena coleoptile

↓

Placed on agar block

↓

Auxin diffuses into agar block

↓

Placed the agar block Asymmetrically On cut coleoptile stump

↓

Coleoptile showed typical curvature

IBA : Indole Butyric Acid

NAA : Naphthalene Acetic acid

MENA: Methyl ester of Naphthalene acetic acid

MCPA: 2 Methyl 4 chloro phenoxy acetic acid

TIBA : 2, 3, 5 Tri iodo benzoic acid

2, 4-D : 2, 4 dichloro phenoxy acetic acid

2, 4, 5-T: 2, 4, 5 – Trichloro phenoxy acetic acid

Natural auxins may occur in the form of either *free auxins*- which freely move or diffuse out of the plant tissues readily or *bound auxins*- which are released from plant tissues only after hydrolysis, autolysis or enzymolysis.

Physiological effects of auxin

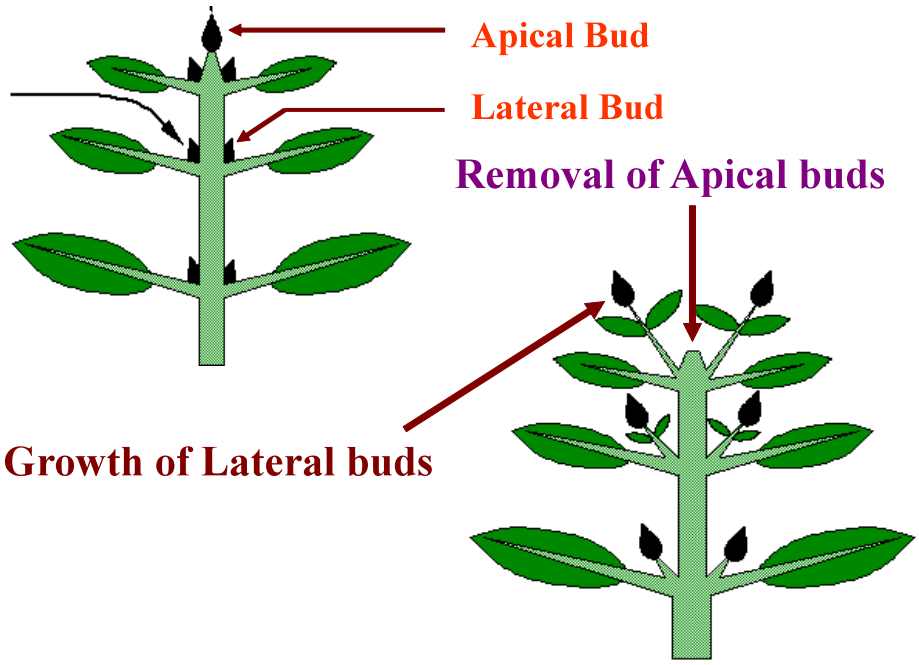
1. Cell division and elongation

The primary physiological effects of auxin are cell division and cell elongation in the shoots. It is important in the secondary growth of stem and differentiation of xylem and phloem tissues.

2. Apical dominance

In many plants, if the terminal bud is intact and growing, the growth of lateral buds just below it remains suppressed. Removal of the apical bud results in the rapid growth of lateral buds. This phenomenon in which the apical bud dominates over the lateral buds and does not allow the lateral buds to grow is known as *apical dominance*.

Skoog and Thimmann (1948) pointed out that the apical dominance might be under the control of auxin produced at the terminal bud and which is transported downward through the stem to the lateral buds and hinders the growth. They removed the apical bud and replaced it with *agar* block. This resulted in rapid growth of lateral buds. But when they replaced the apical bud with agar block containing auxin, the lateral buds remained suppressed and did not grow.



3. Root initiation

In contrast to stem, the higher concentration of auxin inhibits the elongation of roots but the number of lateral roots is considerably increased i.e., higher concentration of auxin induces more lateral branch roots. Application of IAA in lanolin paste (lanolin is a soft fat prepared from wool and is good solvent for auxin) to the cut end of a young stem results in an early and extensive rooting. This fact is of great practical importance and has been widely utilized to promote root formation in economically useful plants which are propagated by cuttings.

4. Prevention of abscission

Natural auxins prevent the formation of abscission layer which may otherwise result in the fall of leaves, flowers and fruits.

5. Parthenocarpy

Auxin can induce the formation of parthenocarpic fruits (fruit formation without pollination and fertilization). In parthenocarpic fruits, the concentration of auxin in the ovaries is higher than in the ovaries of plants which produce fruits only after fertilization. In

the later cases, the concentration of the auxin in ovaries increases after pollination and fertilization.

6. Respiration

Auxin stimulates respiration and there is a correlation between auxin induced growth and respiration. Auxin may increase the rate of respiration indirectly through increased supply of ADP by rapidly utilizing ATP in the expanding cells.

7. Callus formation

Besides cell elongation, auxin may also be active in cell division. In many tissue cultures, where the callus growth is quite normal, the continued growth of such callus takes place only after the addition of auxin.

8. Eradication of weeds

Some synthetic auxins especially 2, 4- D and 2, 4, 5-T are useful in eradication of weeds at higher concentrations.

9. Flowering and sex expression

Auxins generally inhibit flowering but in pine apple and lettuce it promotes uniform flowering.

Distribution of auxin in plants

In plants, auxin (IAA) is synthesized in growing tips or meristematic regions from where; it is transported to other plant parts. Hence, the highest concentration of IAA is found in growing shoot tips, young leaves and developing auxiliary shoots. In monocot seedling, the highest concentration of auxin is found in coleoptile tip which decreases progressively towards its base.

In dicot seedlings, the highest concentration is found in growing regions of shoot, young leaves and developing auxiliary shoots. Within the plants, auxin may present in two forms. i.e., *free auxins* and *bound auxins*. Free auxins are those which are easily extracted by various organic solvents such as diethyl ether. Bound auxins on the other hand, need more drastic methods such as hydrolysis, autolysis, enzymolysis etc. for extraction of auxin. Bound

auxins occur in plants as complexes with carbohydrates such as glucose, arabionse or sugar alcohols or proteins or amino acids such as aspartate, glutamate or with inositol.

Biosynthesis of auxin (IAA) in plants

Thimann (1935) found that an amino acid, tryptophan is converted into Indole 3 acetic acid. Tryptophan is the primary precursor of IAA in plants. IAA can be formed from tryptophan by two different pathways.

1. By deamination of tryptophan to form indole-3-pyruvic acid followed by decarboxylation to from indole-3-acetaldehyde. The enzymes involved are tryptophan deamintion and indole pyruvate decarboxylase respectively.
2. By decarboxylation of tryptophan to form tryptamine followed by deamination to form indole-3-acetaldehyde and the enzymes involved are tryptophan decarboxylase and tryptamine oxidase respectively. Indole 3-acetaldehyde can readily be oxidized to indole 3-acetic acid (IAA) in the presence of indole 3-acetaldehyde dehydrogenase.

Transport of auxin in plant

The transport of auxin is predominantly polar. In stems, polar transport of auxin is basipetal i.e., it takes place from apex towards base. Polar transport of auxin is inhibited by 2, 3, 5 Triiodobenzoic acid (TIBA) and Naphthyl thalamic acid (NPA). The substances are called as antiauxins.

Destruction / Inactivation of auxin in plants

Auxin is destructed by the enzyme IAA oxidase in the presence of O_2 by oxidation.

$$IAA + H_2O_2 + O_2 \xrightarrow{\text{IAA Oxidase}} \text{3-methylene oxindole} + H_2O + CO_2$$

Rapid inactivation may also occur by irradiation with x-rays and gamma rays. UV light also reduces auxin levels in plants. Inactivation or decomposition of IAA by light has been called as photo oxidation.

Mechanism of Action

IAA increases the plasticity of cell walls so that the cells stretch easily in response to turgor pressure. It has been suggested that IAA acts upon DNA to influence the production of mRNA. The mRNA codes for specific enzymes responsible for expansion of cell walls. Recent evidences indicate that IAA increases oxidative phosphorylation in respiration and enhanced oxygen uptake. The growth stimulation might be due to increased energy supply and it is also demonstrated that auxin induces production of ethylene in plants.

Gibberellins

Discovery

A Japanese scientist Kurosawa found that the rice seedlings infected by the fungus *Gibberella fujikuroi* grow taller and turned very thin and pale. An active substance was isolated from the infected seedlings and named as Gibberellin.

Biosynthesis of gibberellins in plants

The primary precursor for the formation of gibberellins is acetate.

Acetate + COA → Acetyl COA → Mevalonic acid → MA pyrophosphate → Isopentanyl pyrophosphate → Geranyl pyrophosphate → GGPP → Kaurene → Gibberellins.

Physiological effects of gibberellins

1. Seed germination

Certain light sensitive seeds eg. Lettuce and tobacco show poor germination in dark. Germination starts vigorously if these seeds are exposed to light or red light. This requirement of light is overcome if the seeds are treated with gibberellic acid in dark.

2. Dormancy of buds

In temperature regions the buds formed in autumn remain dormant until next spring due to severe cold. This dormancy of buds can be broken by gibberellin treatments. In potato also, there is a dormant period after harvest, but the application of gibberellin sprouts the refer vigorously.

3. Root growth

Gibberellins have little or no effect on root growth. At higher concentration, some inhibition of root growth may occur. The initiation of roots is markedly inhibited by gibberellins in isolated cuttings.

4. Elongation of internodes

The most pronounced effect of gibberellins on the plant growth is the elongation of the internodes. Therefore in many plants such as dwarf pea, dwarf maize etc gibberellins overcome the genetic dwarfism.

5. Bolting and flowering

In many herbaceous plants, the early period of growth shows rosette habit with short stem and small leaves. Under short days, the rosette habit is retained while under long days bolting occurs i.e. the stem elongates rapidly and is converted into polar axis bearing flower primordia. This bolting can also be induced in such plants by the application of gibberellins even under non-inductive short days.

In *Hyoscyamus niger* (a long day plant) gibberellin treatment causes bolting and flowering under non-inductive short days. While in long day plants the gibberellin treatment usually results in early flowering. In short day plants, its effects are quite variable. It may either have no effect or inhibit or may activate flowering.

6. Parthenocarpy

Germination of the pollen grains is stimulated by gibberellins; likewise, the growth of the fruit and the formation of parthenocarpic fruits can be induced by gibberellin treatment. In many cases, eg. pome and stone fruits where auxins have failed to induce parthenocarpy, the gibberellins have proven to be successful. Seedless and fleshly tomatoes and large sized seedless grapes are produced by gibberellin treatments on commercial scale.

7. Synthesis of the enzyme α - amylase

One important function of gibberellins is to cause the synthesis of the enzyme α - amylase in the aleurone layer of the endosperm of cereal grains during germination. This enzyme brings about hydrolysis of starch to form simple sugars which are then translocated to growing embryo to provide energy source.

Distribution of gibberellins in plant

Gibberellins are found in all parts of higher plants including shoots, roots, leaves, flower, petals, anthers and seeds. In general, reproductive parts contain much higher concentrations of gibberellins than the negative parts. Immature seeds are especially rich in gibberellins (10-100 mg per g fresh weight).

In plants, gibberellins occur in two forms free gibberellins and bound gibberellins. Bound gibberellins usually occur as gibberellin – glycosides.

CYTOKININS (Kinetin)

Kinetin was discovered by Skoog and Miller (1950) from the tobacco pith callus and the chemical substance was identified as 6-furfuryl aminopurine. Because of its specific effect on *cytokinesis* (cell division), it was called as cytokinins or kinetin. The term, cytokinin was proposed by Letham (1963). Fairley and Kingour (1966) used the term, *phytokinins* for cytokinins because of their plant origin. Chemically cytokinins are kinins and they are purine derivatives.

Cytokinins, besides their main effect on cell division, also regulate growth and hence they are considered as natural plant growth hormones. Some of the very important and commonly known naturally occurring cytokinins are Coconut milk factor and Zeatin. It was also identified that cytokinin as a constituent of t-RNA.

Naturally occurring cytokinins

Cytokinins can be extracted from coconut milk (liquid endosperm of coconut), tomato juice, flowers and fruits of *Pyrus malus*; fruits of *Pyrus communis* (Pear), *Prunus cerasiferae* (plum) and *Lycopersicum esculentum* (bhendi); Cambial tissues of *Pinus radiata*, *Eucalyptus regnans* and *Nicotiana tabacum*; immature fruits of *Zea mays*, *Juglans* sp. and *Musa* sp; female gametophytes of *Ginkgo biloba*; fruitlets, embryo and endosperms of *Prunus persica*; seedling of *Pisum sativum*; root exudates of *Helianthus annuus* and tumour tissues of tobacco. According to Skoog and Armstrong (1970), at least seven well established types of cytokinins have been reported from the plants.

Biosynthesis

It is assumed that cytokinins are synthesised as in the case of purines in plants (nucleic acid synthesis). Root tip is an important site of its synthesis. However, developing seeds and cambial tissues are also the site of cytokinin biosynthesis. Kende (1965) reported that cytokinins move upwards perhaps in the xylem stream. However, basipetal movement in petiole and isolated stems are also observed. Seth *et al* (1966) found that auxin enhances kinetin movement (translocation) in bean stems.

Physiological effects of cytokinins

1. Cell division

The most important biological effect of kinetin on plants is to induce cell division especially in tobacco pith callus, carrot root tissue, soybean cotyledon, pea callus etc.

2. Cell enlargement

Like auxins and gibberellins, the kinetin may also induce cell enlargement. Significant cell enlargement has been observed in the leaves of *Phaseolus vulgaris*, pumpkin cotyledons, tobacco pith culture, cortical cells of tobacco roots etc.

3. Concentration of apical dominance

External application of cytokinin promotes the growth of lateral buds and hence counteracts the effect of apical dominance

4. Dormancy of seeds

Like gibberellins, the dormancy of certain light sensitive seeds such as lettuce and tobacco can also be broken by kinetin treatment.

5. Delay of senescence (Richmand - Lang effect)

The senescence of leaves usually accompanies with loss of chlorophyll and rapid breakdown of proteins. Senescence can be postponed to several days by kinetin treatment by improving RNA synthesis followed by protein synthesis.

Richmand and Lang (1957) while working on detached leaves of *Xanthium* found that kinetin was able to postpone the senescence for a number of days.

6. Flower induction

Cytokinins can be employed successfully to induce flowering in short day plants.

7. Morphogenesis

It has been shown that high auxin and low kinetin produced only roots whereas high kinetin and low auxin could promote formation of shoot buds.

8. Accumulation and translocation of solutes

Plants accumulate solutes very actively with the help of Cytokinin and also help in solute translocation in phloem.

9. Protein synthesis

Osborne (1962) demonstrated the increased rate of protein synthesis due to translocation bys kinetin treatment.

10. Other effects

Cytokinins provide resistance to high temperature, cold and diseases in some plants. They also help in flowering by substituting the photoperiodic requirements. In some cases, they stimulate synthesis of several enzymes involved in photosynthesis.

11. Commercial applications

Cytokinins have been used for increasing shelf life of fruits, quickening of root induction and producing efficient root system, increasing yield and oil contents of oil seeds like ground nut.

Ethylene

Ethylene is the only natural plant growth hormone exists in gaseous form.

Important physiological elects

1. The main role of ethylene is it hastens the ripening of fleshy fruits eg. Banana, apples, pears, tomatoes, citrus etc.
2. It stimulates senescence and abscission of leaves
3. It is effective in inducing flowering in pine apple
4. It causes inhibition of root growth
5. It stimulates the formation of adventitious roots
6. It stimulates fading of flowers
7. It stimulates epinasty of leaves.

Abscisic acid

Addicott (1963) isolated a substance strongly antagonistic to growth from young cotton fruits and named Abscissin II. Later on this name was changed to Abscisic acid. This substance also induces dormancy of buds therefore it also named as Dormin.

Abscisic acid is a naturally occurring growth inhibitor.

Physiological effects

The two main physiological effects are

1. Geotropism in roots
2. Stomatal closing
3. Besides other effects

1. Geotropism in roots

Geotropic curvature of root is mainly due to translocation of ABA in basipetal direction towards the root tip.

2. Stomatal closing

ABA is synthesized and stored in mesophyll chloroplast. In respond to water stress, the permeability of chloroplast membrane is lost which resulted is diffusion of ABA out of chloroplast into the cytoplasm of the mesophyll cells. From mesophyll cells it diffuses into guard cells where it causes closing of stomata.

3. Other effects

i. Including bud dormancy and seed dormancy

ii. Includes tuberisation

iii. Induces senescence of leaves fruit ripening, abscission of leaves, flowers and fruits

iv. Increasing the resistance of temperate zone plants to frost injury.

Growth retardants

There is no. of synthesis compounds which prevent the gibberellins from exhibiting their usual responses in plants such as cell enlargement or stem elongation. So they are called as anti gibberellins or growth retardants. They are

1. Cycocel (2- chloroethyl trimethyl ammonium chloride (CCC)
2. Phosphon D – (2, 4 – dichlorobenzyl – tributyl phosphonium chloride)
3. AMO – 1618
4. Morphactins

5. Maleic hydrazide

Explant Preparation

Lab 9

L. Amal Alayedh

L. Azzah Tayeb

Explant

The explant is a piece of plant tissue placed into tissue culture. Explant isolated from the tissues of higher plants and brought into culture. Like excised organs, require a nutrient medium consisting of mineral salts mixture, a carbon source, (usually sucrose) and vitamins. In addition phytohormons (auxins and cytokinins), or their synthetic counterparts, are required to initiate and maintain cell division; occasionally other organic supplements, for instance amino acids or hexitols, are necessary to ensure the prolonged growth of the excised tissue to give an established callus.

To selection explant there are some factors that must be considered are as follows:

1- Physiological or ontogenic age of the organ that is to serve the explant source.

2- Season in which the explant is obtained.

3- Size and location of the explant.

4- Quality of the source plant.

5- Ultimate goal of cell culture.

6- Plant genotype.

1- Explant age:

The age of the explant is very important. Physiologically younger tissue is more responsive in vitro, usually the newest formed and is easier to surface disinfect and establish clean cultures. While, older tissue will not form callus that is capable of regeneration.

2- Season:

The season of the year can be effects on contamination and response in culture. For example, during the spring of the year buds or shoots taken are more responsive.

3- Explant size:

The size of the explant has an effect on the response of the tissue. The smaller explant harder to cultuer wherese the medium of culture has to have additional components. Subsequently, the large explants probably contain more nutrient reserves and plant growth regulators to sustain the culture.

4- Plant Quality:

It is best to obtain explants from healthy plants compared to plants under nutritional or water stress or plants which are exhibiting disease symptoms.

5- Goal:

The choice of explant tissue will vary depending on what type of a response is desired from the cell culture. For instance:

(a) if the goal is clonal propagation, then the explant will be a lateral or terminal shoot or bud.
(b) If callus induction is the goal, then pieces of the cotyledon, hypocotyl, stem, leaf, or embryo are used.
(c) For protoplast isolation, leaf tissue from aseptically germinated seed is a good source.

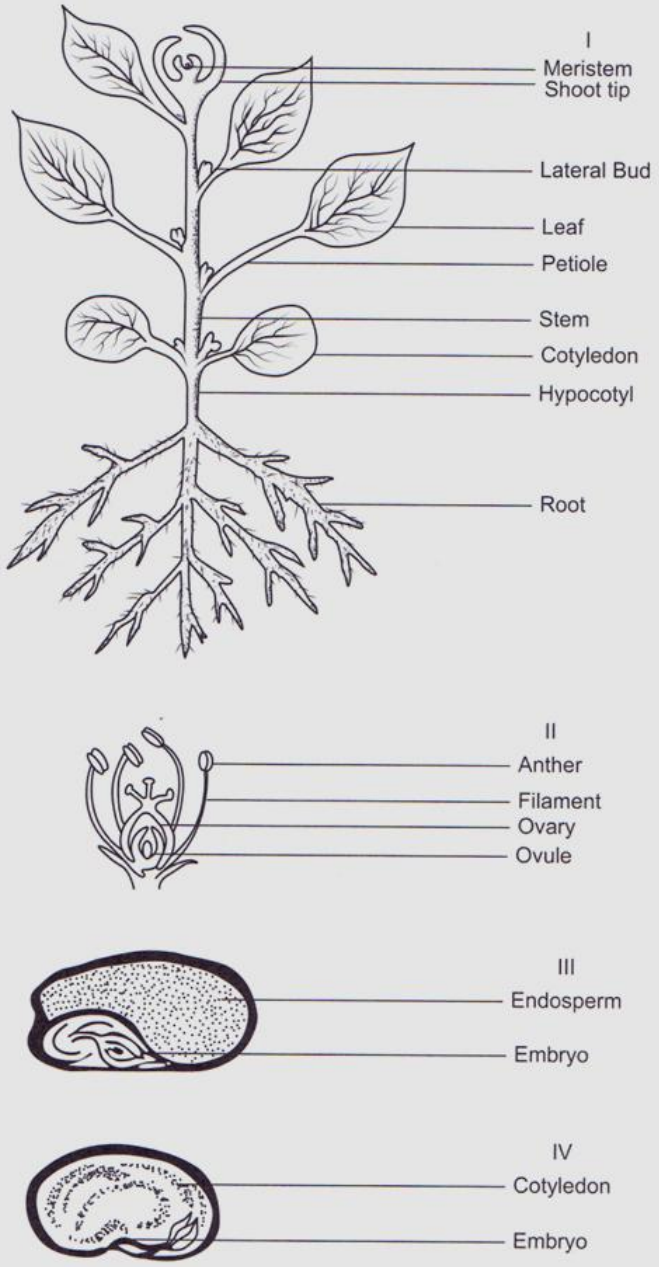


FIGURE 4.1 Schematic drawings (from top to bottom) of a plant, a flower, and monocotyledenous and dicotyldenous seeds indicate potential explant tissues.

Explant: 1- Embryo and Organ Culture
Embryos of maize (Zea mays)

The removal and culture of embryos of higher plants was one of the earliest successful techniques in plant tissue and organ culture.

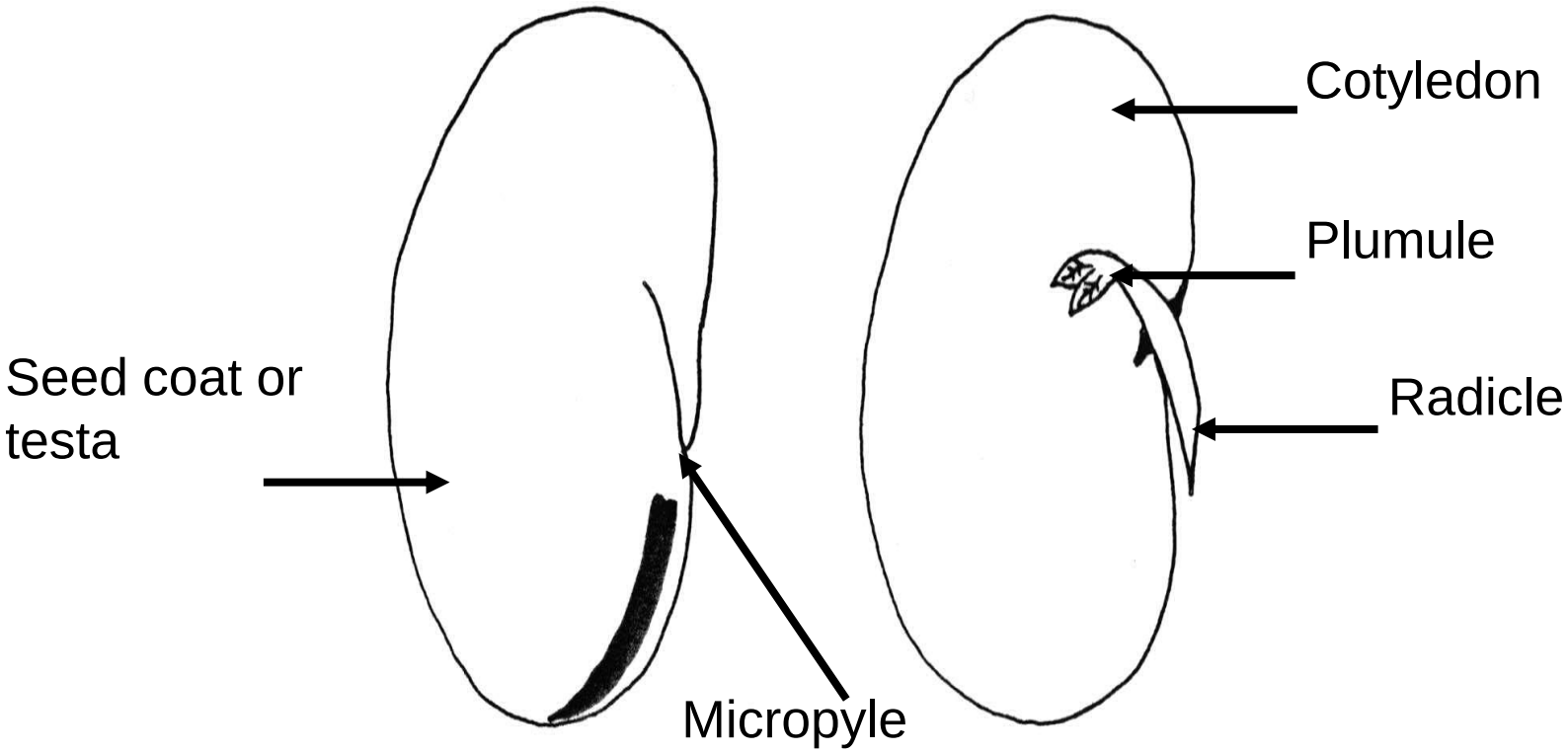
Embryos removed from the seeds of Shepherd's Purse (Capsella bursa-pastoris) can be cultured under completely aseptic conditions.

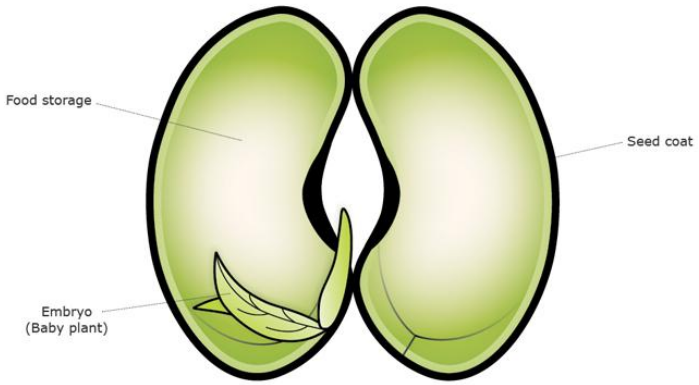
Over the years the excised embryos of many species have been brought into culture using relatively simple nutrient media.

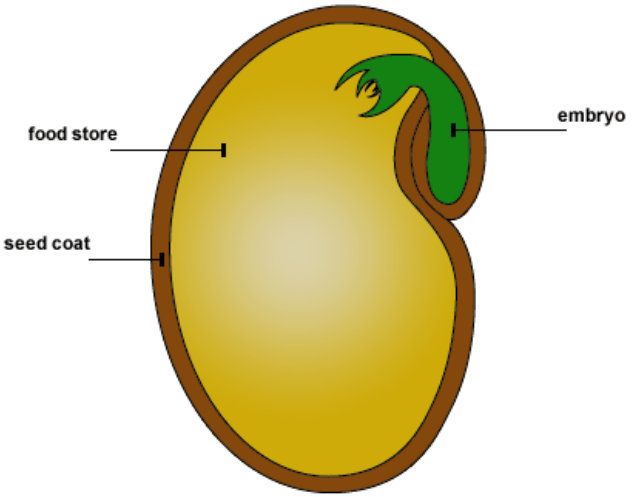
The advantages of growing an embryo isolated from the rest of the seed

The advantages of growing an embryo isolated from the rest of the seed, apart from the intrinsic interest in doing so, are to remove the immature plant from the endosperm and/or cotyledon(s) which may in particular cases prevent or modify the development of the plant.

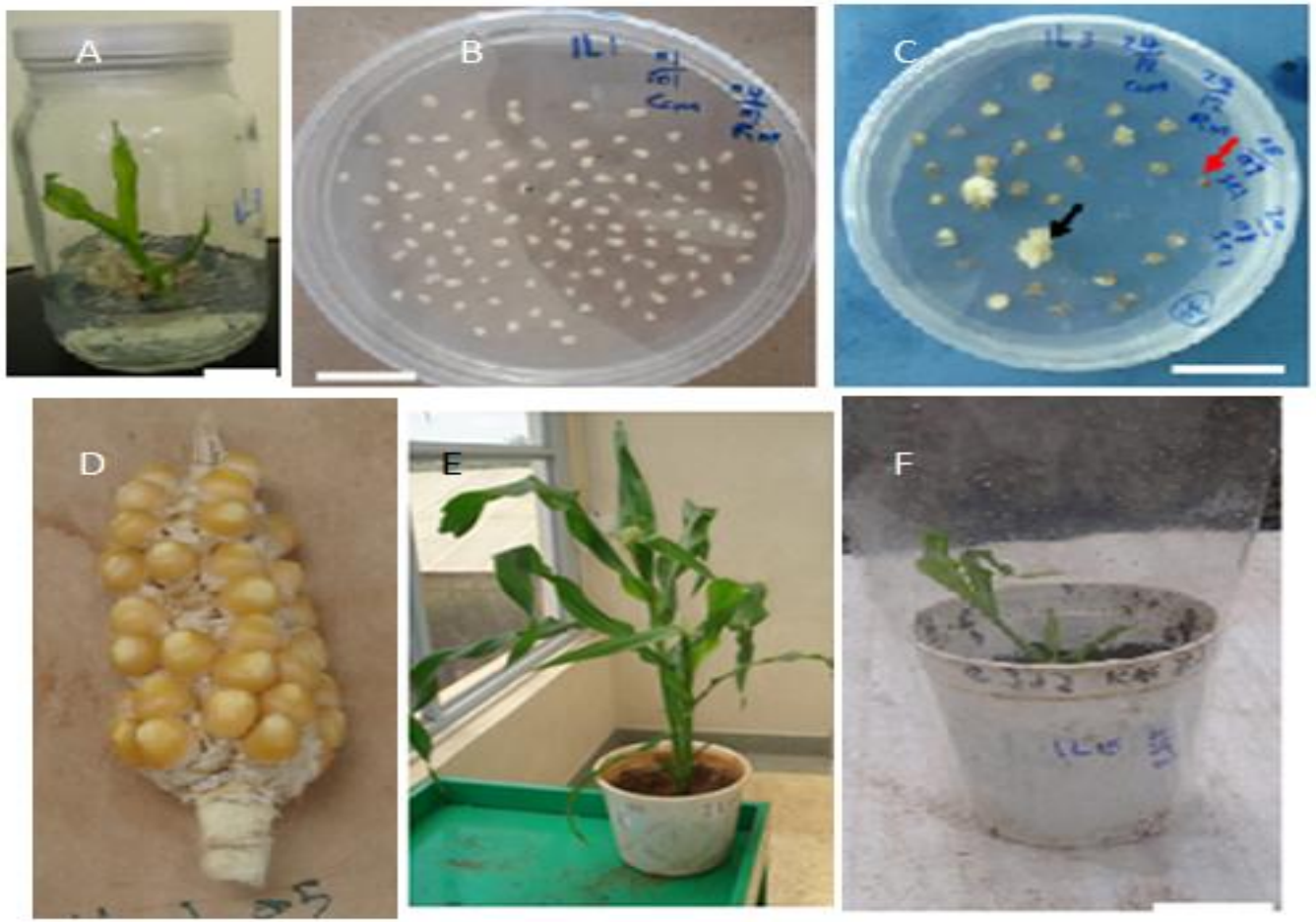
In certain instances the excised embryo can also be used as a means of propagating species which resist attempts to use standard methods of vegetative propagation. In this experiment the subject, an embryo of maize, is large, easy to remove from the grain, and can be brought into sterile culture easily and successfully.





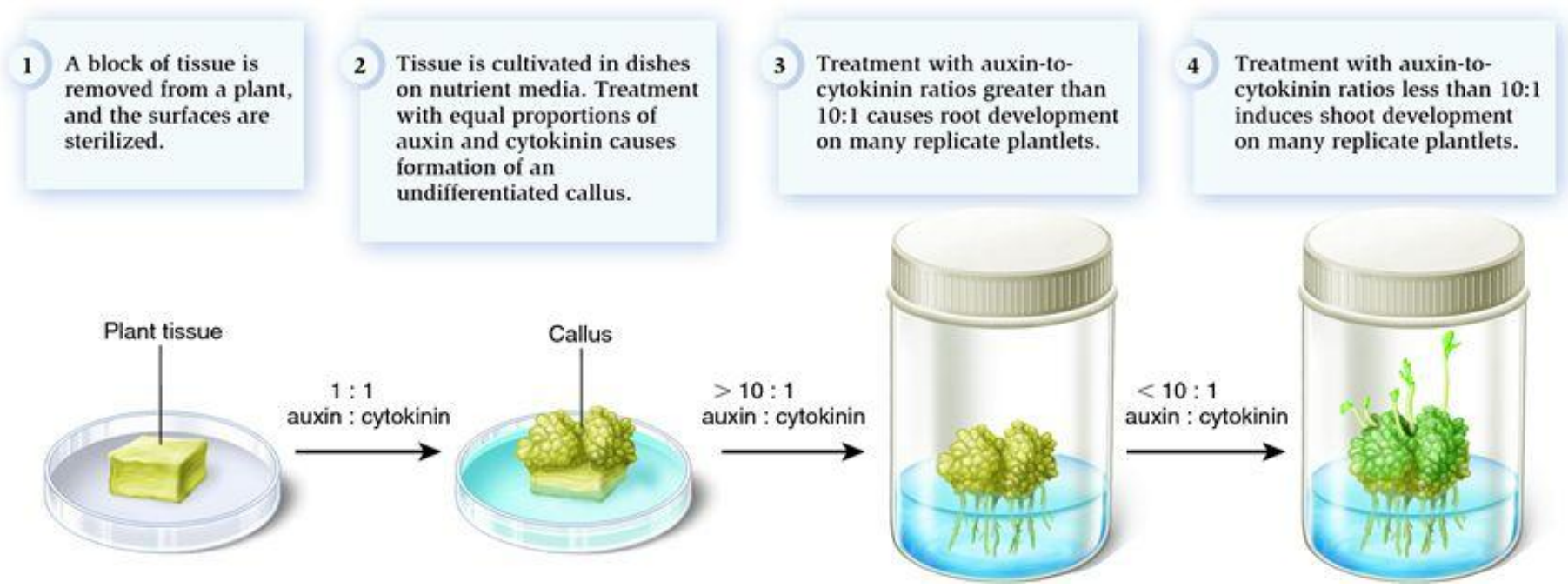


Embryo and Organ Culture; Embryos of maize (Zea mays)



2- Isolation of Explants, Establishment and Maintenance of Callus (*Daucus carota*)

- An explant can develop a callus as a wound response that consists of unorganized, friable, large, vacuolated, dividing cells that are highly differentiated.
- Callus also can be produced without wounding by germinating of seeds on a plant medium containing growth regulator. The cells of callus are vary in size, shape, pigmentation and in genetic expression.



Plant tissue culture illustrates the impact of different proportions of auxin and cytokinin on plant organ development.

Purpose: To gain experience in aseptic technique and callus induction from varied explants (seedling, fruit, root).

Materials and Equipments:

-MS medium
- Cotton
- glass petri dishes
- Stirile distilled water contained in Erlenmeyer conical flasks
- sheets of aluminium foil
- Forceps
- Scalpels

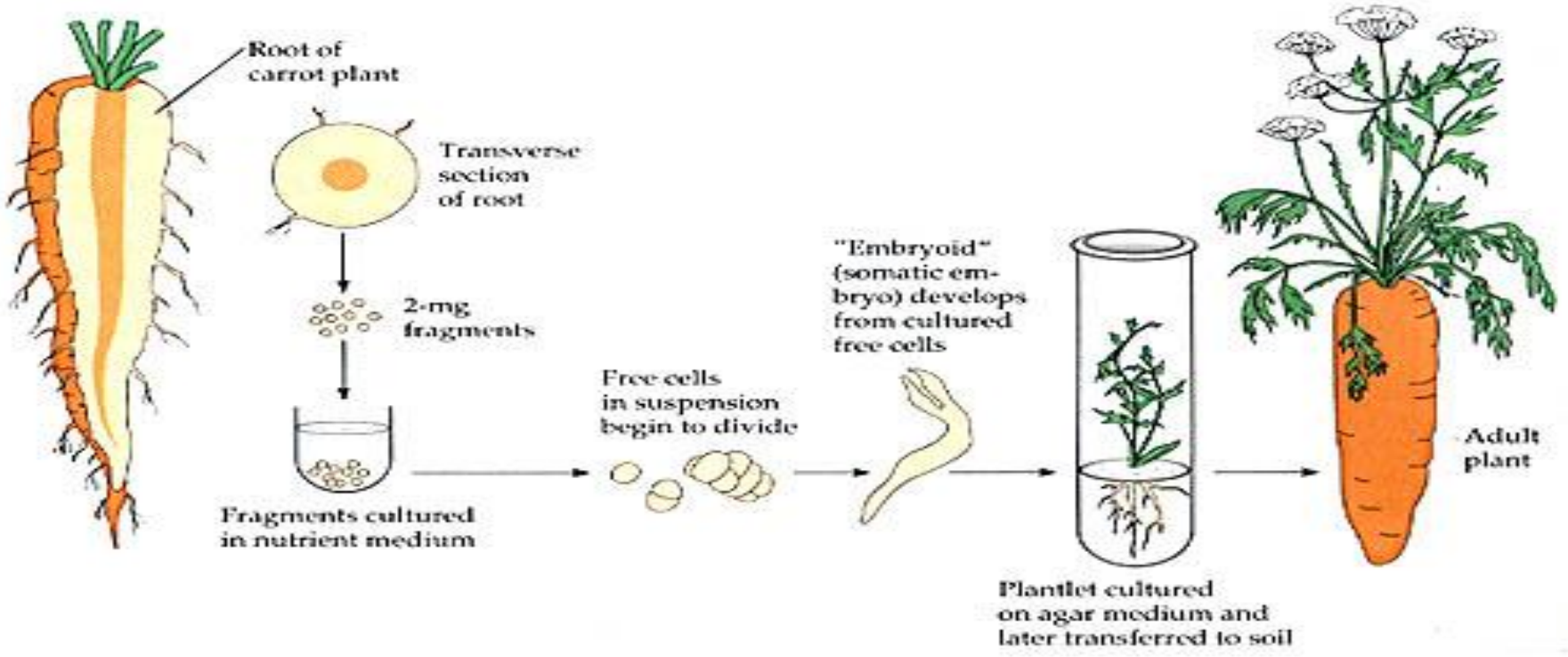
Non sterile items

- Tap root of carrot at least 200 mm in length and 40 mm in diameter.
- racks, preferably plastic or metal to hold 12 culture tubes
- 1000 ml of a solution of sodium hypochlorite approximately 20% (v/v)
- waterproof marking pen
- Glass beaker
- analytical balance
- bunsen or ethanol burner
- Erlenmeyer flask containing 100ml 95% ethanol
- roll parafilm

Experiments Procedures

1- Reject all diseased, damaged, or irregularly shaped individuals. Scrub the carrots under running tap water to remove all surface detritus using a brush.
2- Cut the root into ½- inch sections and surface sterilization in 15% chlorine bleach for 10-15 min.
3- Transfer sterilized carrots to the sterile room marking sure the UV lights are switched off before entering. Powerful UV rays are harmful to the eyes and skin! After wiping it clean with 70% ethanol set out the working.
3- Rinse three times in sterile distilled water to completely remove the hypochlorite.
4- Cut off tissue burned by chlorine bleach and culture.
5- Place all cultures in the dark and incubate in the dark at 27 – 30° C.

Isolated of Plant Material and Studies on Growth and Cell Division Experiment: Isolation of Explants, Establishment and Maintenance of Callus (*Daucus carota*)



Observations:

Record cultures observations once a week over a 6-week period. It is include notes on culture conditions, callus formation, and contamination.

Isolation, Culture and Fusion of Protoplasts from Higher Plants

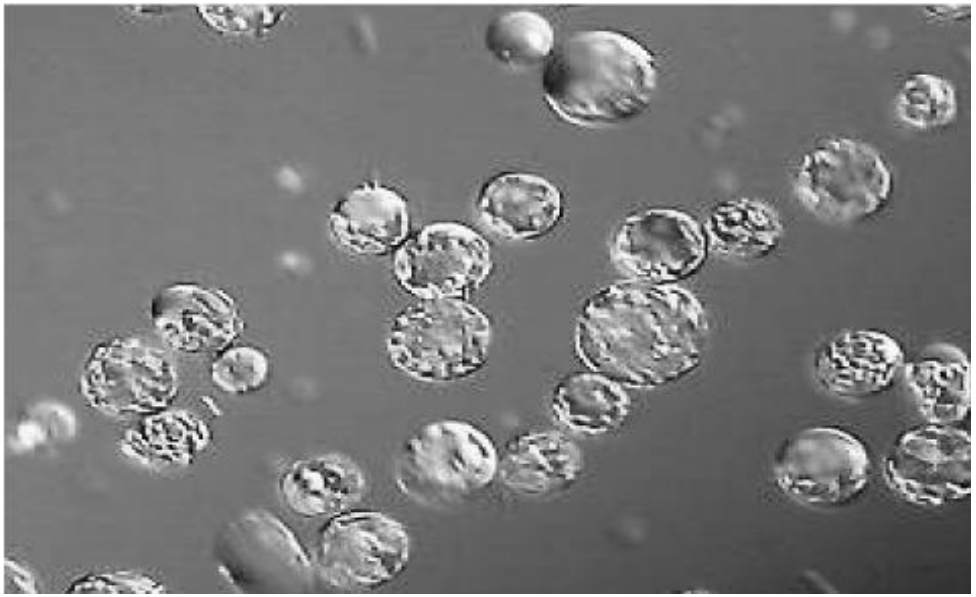
** Isolation and Culture of Mesophyll Protoplasts from Tobacco Leaves

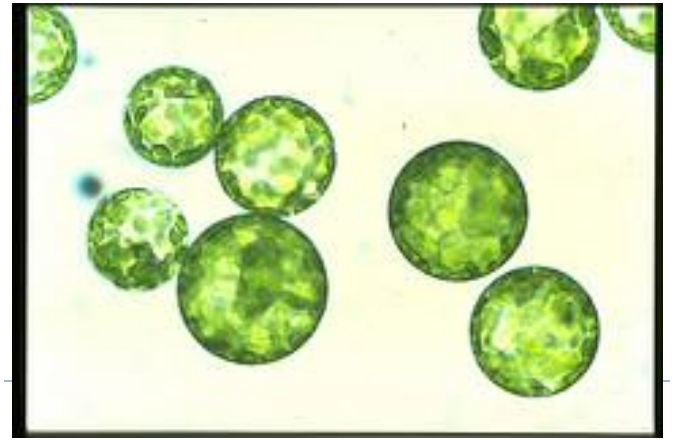
- Isolated plant protoplasts are cells from which the wall has been removed either mechanically or enzymically.

Protoplast preparation was at first done in two steps:

1. At first was the middle lamella dissolved by pectinases.
2. then was the cell wall broken down by cellulase .

Isolation, Culture and Fusion of Protoplasts from Higher Plants (Isolation and Culture of Mesophyll Protoplasts from Tobacco Leaves)





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Thank
you!

GURU NANAK COLLEGE (AUTONOMOUS)
VELACHERY- CHENNAI – 42.

- DEPARTMENT OF PLANT BIOLOGY AND PLANT BIOTECHNOLOGY
- SEMESTER V
- COURSE: PLANT BIOTECHNOLOGY
- COURESE CODE:16UPBTC09

BY Dr.E.GAYATHIRI

Basic requirements of a tissue culture laboratory

Preparation area, Transfer area, Culture area, Acclimatization area

Transfer area

Laminar air flow bench, Disinfectants, Other sanitary aids (glows, masks etc.)

Culture area

Incubators, Shakers, Controlled light, temperature RH etc.

Acclimatization area

Facility to provide different shade levels

Basic requirements of a tissue culture laboratory

- **Callus Culture:**
- Callus is formed by the proliferation of the parent tissue. The cells of a callus are parenchymatous, amorphous and unorganised. Generally callus is formed as a result of injury at the cut ends of a stem or a root.
- **Materials:**
- Callus may initiate from explants of any multicellular plant. Explants from stem, root, leaf, flower, fruit or seed etc. may be taken for culture.
- Callus formation has been recorded from storage parenchyma, pericyclic cells of roots, cambial cells of vascular bundles, provascular cells, secondary phloem, pith cells, mesophyll cells and cotyledons.
- **Nutrient Medium of Callus Culture:**
- Some standard media, such as, Murashige and Skoog's medium can be successfully used for callus culture. For initiation and maintaining callus kinetin is widely used in the medium.
- For callus initiation usually an exogenous supply of hormone is required.

GROWTH HORMONES

- **These are:**
- (a) Auxin requiring cultures,
- (b) Cytokinin requiring cultures,
- (c) Cultures requiring both auxin and cytokinin,
- (d) Gibberellin requiring cultures.

Methods of Callus Culture:

- Usually explants from suitable materials (such as, carrot root, potato or sweet potato tuber, stem of tobacco, hypocotyl and cotyledon of soya bean etc..) are taken.
- The explants is first surface sterilised with 1.6% sodium hypochlorite solution or 0.1% mercuric chloride solution or 1% aqueous solution of bromine.
- Then the inner uncontaminated tissue is excised. If the excised tissue (such as, root, hypocotyl, cotyledon etc.) is taken from a seedling then the seed before germination is surface sterilised and allowed to germinate under aseptic conditions.

Carrot Root Culture:

- (1) Fresh and healthy carrot root is selected. It is thoroughly washed in running tap water.
- (2) External 1-2 mm is scraped. Upper 1 cm of carrot root is discarded and then it is cut into 0.5 cm thick slices (Fig. 19).
- (3) These slices are placed immediately in a beaker containing water.
- (4) These are then transferred to a beaker containing sodium hypochlorite solution and kept there for 10 minutes.
- (5) Slices are taken out with a sterile force from the hypochlorite solution and washed successively in 3 beakers containing double distilled water keeping the slices for 20—30 seconds in each. The slices are kept in the third beaker.

Carrot Root Culture: CONT.

- 5) Slices are taken out with a sterile force from the hypochlorite solution and washed successively in 3 beakers containing double distilled water keeping the slices for 20—30 seconds in each. The slices are kept in the third beaker.
- A carrot slice is taken and is placed on a petridish. Tissue cylinders are cut out from the cambial region by a sterilised cork borer, after cutting maximum number of tissue cylinders from the cambial region remaining portion of the slice is discarded
- (7) Tissue cylinders are placed in a petridish containing double distilled water.
- (8) A tissue cylinder is transferred to a petridish and its two sides are trimmed with a sterile scalpel and discarded.

Carrot Root Culture: CONT.

- (9) Remaining cylinder is cut into explants measuring 5 mm diameter and 2 mm thickness.
- (10) These explants are placed in a petridish containing double distilled water. (11) Explants are then transferred with a sterile forceps on the surface of a sterile filter paper on a petridish. The upper and lower surfaces of each explants are blotted.
- (12) One such explants is transferred to each culture tube containing the nutrient medium.
- (13) Culture tubes are kept in a glass storage jar, wrapped in aluminium foil and placed in an incubator at 25°C.

Carrot Root Culture: CONT.

- (14) The surface of the explants after few days becomes somewhat rough, indicating initiation of the callus. Callus can be maintained from few weeks to three months depending on the rate of growth.
- (15) Generally after 6-8 weeks the callus is sub-cultured. The callus is divided into small parts of 100 mg approximately.
- (16) Each piece is transferred to a new flask containing 30 c.c. of culture medium and sub-cultured at a temperature of 25°C or above.

Development of a Callus Culture:

- **Callus formation from an explants occurs in three stages:**
- **(a) Induction stage:**
- Metabolism is stimulated and the cells prepare to divide. Cell size remains unchanged.
- **(b) Cell division stage:**
- Cells divide actively and the cell size decreases. Cell division is mainly periclinal and occurs towards the periphery giving rise to wound cambial cells.

Development of a Callus Culture:

- **(c) Differentiation:**
- Cells differentiate by expansion and maturation. Rapidly growing calluses are more or less alike but as the growth rate decreases the calluses show their characteristic structures and forms. But all calluses have some similarities. They all contain nodular or sheet meristems in groups or scattered throughout the tissue.
- **Nature of Callus Tissue:**
- Morphological nature of the callus tissue are hard and anatomically consists of compactly arranged small cells without intercellular spaces. Such callus may be composed of lignified cells.

Micro propagation involves the following steps.

- Selection of plant material
- Washing of plant material
- Media preparation
- Sterilization
- Inoculation
- Culture room
- Transfer of plantlets from test tube flask to pot
- Hardening
- Field trial

ADVANTAGES OF MICROPROPAGATION :

- The basic advantages of micro propagation in forestry are
- • To capture and multiply unique genotypes without the problem of variation , which is inherent in the sexually propagated material.
- • To produce the crop that is uniform and predictable which is not possible through seedlings.
- • Large number of plants can be produced from a single piece of plant part
- • Rapid multiplication of desirable and rare plants can be done.
- • Large number of haploid and homozygous plants can be developed.
- • In-vitro multiplication can be continued through out the year irrespective of season.
- • Disease/ Virus free plants can be produced through tissue culture.