

SARDAR PATEL UNIVERSITY
B. Sc. (BOTANY) Sem. : VI Code: US06CBOT22 (T)
ANATOMY OF ANGIOSPERMS
Total Credit: 4 (Four Lectures per week)
(Total Marks 100, Internal-30 marks, External 70-marks)

1. Introduction and scope of Plant Anatomy:

Applications in systematics, forensics and pharmacognosy.

Structure and Development of Plant Body: Internal organization of plant body: The three tissue systems, types of cells and tissues.

Development of plant body: Polarity,

Cytodifferentiation and organogenesis during embryonic development

UNIT : 1 Introduction and scope of Plant Anatomy :

A plant is a complex structure that consists of a number of parts which constitute the whole plant. If you learn to identify each individual part, you will gain a much greater understanding as to how the plant works as a whole. This can be helpful to aromatherapists who need to be aware of the part of the plant an essential oil was derived from because there is often a connection between the oils location in a plant and its therapeutic action.

Plant anatomy is the study of **plant** tissues and cells in order to learn more about the way these organisms are constructed and how they work. These studies are very important because they lead to a better understanding of how to care for **plants** and fight **plant** diseases. **Plant anatomy** is also known as phytotomy.

Applications in systematics, forensics and pharmacognosy.

Anatomical characters of vegetative and floral parts of flowering plants have been successfully employed to solve taxonomic problems and for the explanation of phylogenetic relationship.

Anatomical evidence can be useful in systematic in several ways :

1. It can well be exploited taxonomically in the identification of fragmentary, say a piece of wood.
2. When morphological characters prove to be of no help in the preliminary identification of herbarium material, anatomical study may prove helpful.
3. Anatomical data has proved to be very useful in understanding evolutionary trends and interrelationship of taxa at and above the species level and at higher taxonomic categories.
4. They are most useful in determining relationship between different genera, families, orders and other taxonomic categories.

Plant anatomy provides characters such as trichomes, stomata, cuticular pattern, leaf venation, wood **anatomy**, growth rings etc. to aid in species identification and in performing physical matches of evidence.

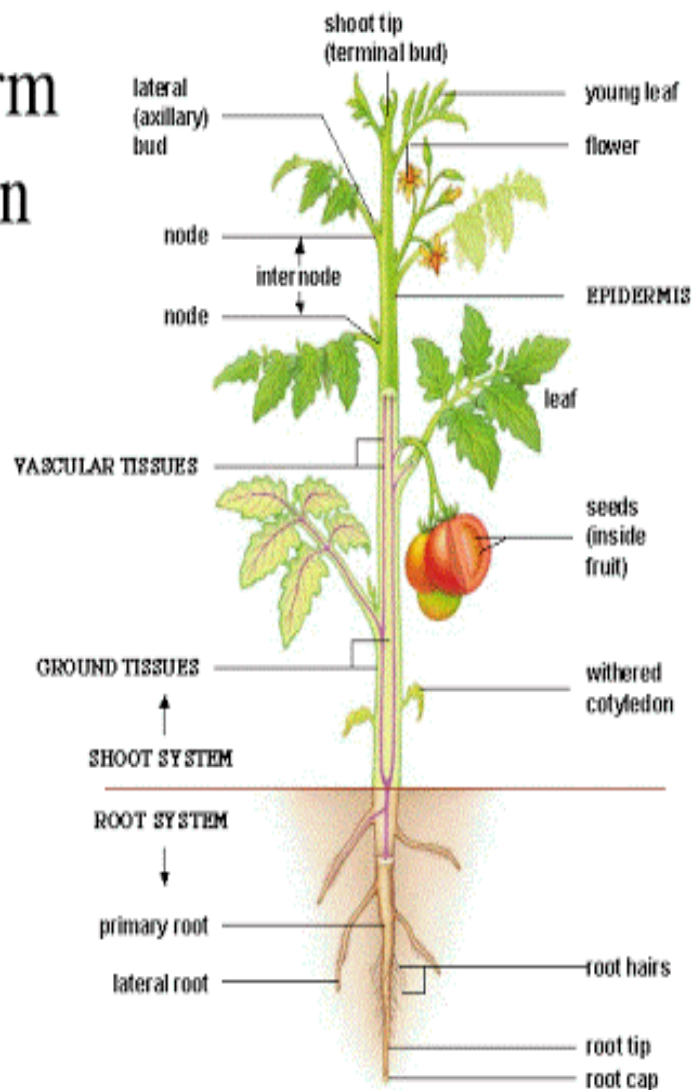
The use of **plant anatomy** can be important as a **forensic** tool in criminal investigations. The knowledge of the preparation of **plant** fragments, the analysis of these fragments, and the interpretation of the data obtained - all must be part of **forensic** botany.

Fragments of herbarium specimens, leaf, dried and powdered medicinal plants etc. The prerequisite of any botanical research is the proper identification of the specimen. Trichome **anatomy**, wood and leaf **anatomy**, leaf epidermis and cuticle etc. provide valuable characters in differentiation between species.

Structure and Development of Plant Body: Internal organization of plant body:

Angiosperm Body Plan

- Ground tissue system
- Vascular tissue system
- Dermal tissue system



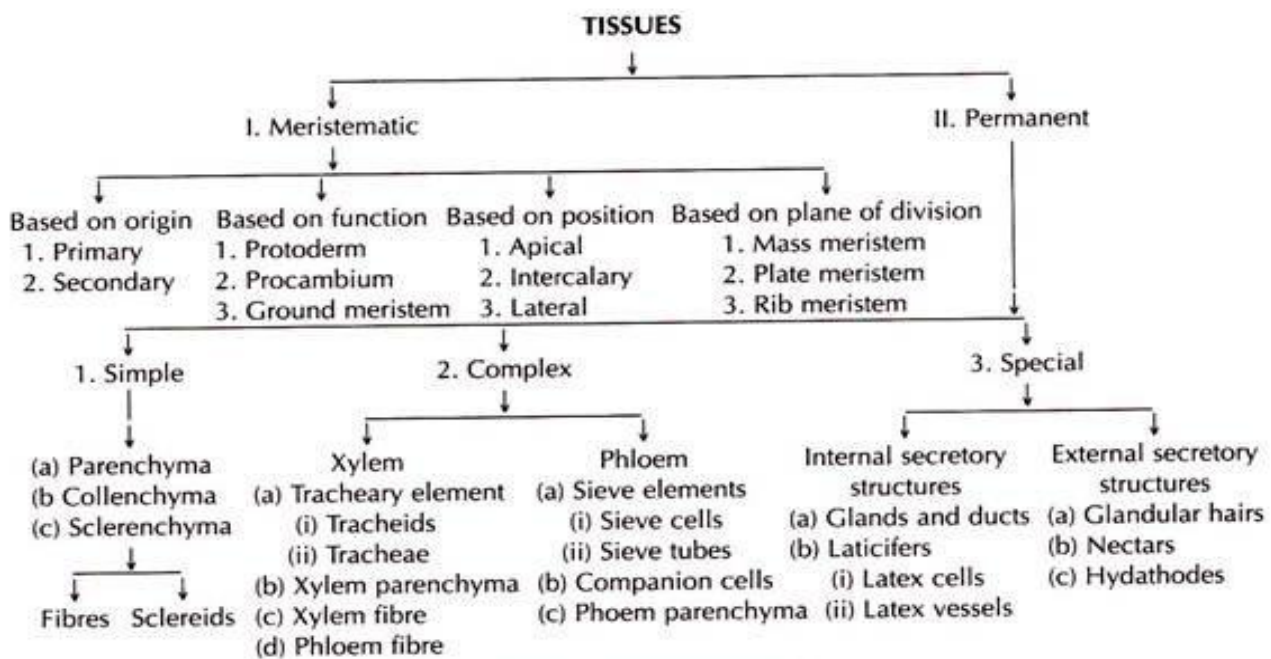


Fig. 5. 38 : Outline classification of tissues

The three tissue systems,

Meristematic Tissue

- The growth of plants occurs only in certain specific regions. This is because the **dividing tissue**, also known as meristematic tissue, is located only at these points.
 - Depending on the region where they are present, meristematic tissues are classified as **apical**, **lateral** and **intercalary**.
 - New cells produced by meristem are initially like those of meristem itself, but as they grow and mature, their characteristics slowly change and they become differentiated as components of other tissues.
1. **Apical meristem** is present at the growing tips of stems and roots and increases the length of the stem and the root.
 2. The girth of the stem or root increases due to **lateral meristem (cambium)**.
 3. **Intercalary meristem** is the meristem at the base of the leaves or internodes (on either side of the node) on twigs.
- As the cells of this tissue are very active, they have **dense cytoplasm**, **thin cellulose walls** and **prominent nuclei**. They **lack vacuoles**.

Permanent Tissue

- What happens to the cells formed by meristematic tissue? They take up a specific role and lose the ability to divide. As a result, they form a permanent tissue.
- This process of taking up a permanent shape, size, and a function is called **differentiation**. Cells of meristematic tissue differentiate to form different types of permanent tissue.
- A few layers of cells form the basic **packing tissue**. This tissue is parenchyma, a type of permanent tissue. It consists of relatively **unspecialised cells with thin cell walls**.

Parenchyma

- They are **live cells**. They are usually loosely packed, so that large spaces between cells (intercellular spaces) are found in this tissue.

Chlorenchyma

- This tissue provides support to plants and also **stores food**. In some situations, it contains chlorophyll and performs photosynthesis, and then it is called chlorenchyma.

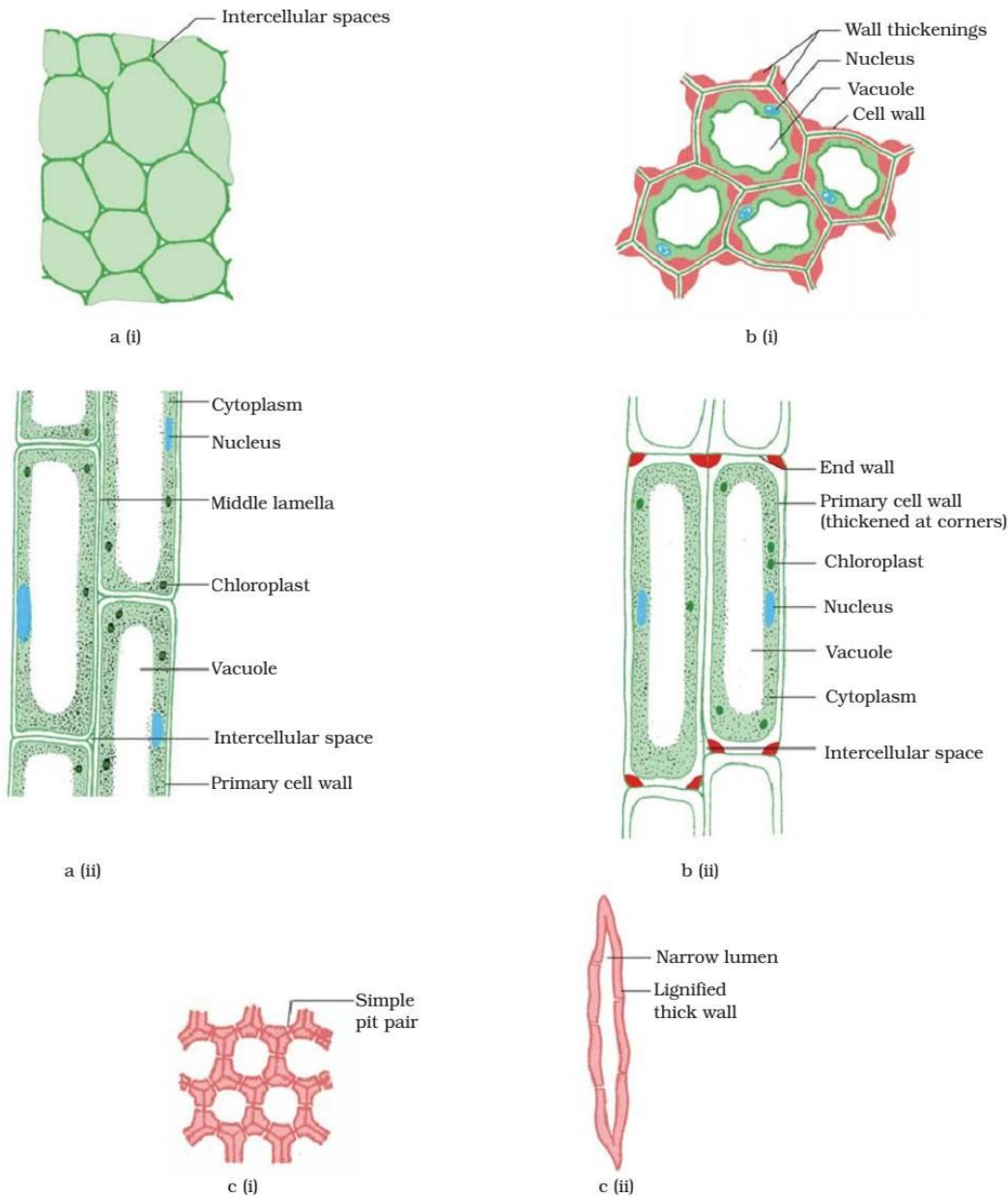
Aerenchyma

- In aquatic plants, large air cavities are present in parenchyma to give **buoyancy** to the plants to help them float. Such a parenchyma type is called aerenchyma. The parenchyma of stems and roots also stores nutrients and water.

Collenchyma

- The flexibility in plants is due to another permanent tissue, collenchyma. It allows easy bending in various parts of a plant (leaf, stem) without breaking. It also provides mechanical support to plants. We can find this tissue in leaf stalks below the epidermis. The cells of this tissue are living, elongated and irregularly thickened at the corners. There is **very little intercellular space**.
- **Sclerenchyma**
- Yet another type of permanent tissue is sclerenchyma. It is the tissue which makes the plant **hard and stiff**. We have seen the husk of a coconut. It is made of sclerenchymatous tissue. The cells of this tissue are **dead**. They are long and narrow as the walls are thickened due to **lignin** (a chemical substance which acts as cement and hardens them). Often these walls are so thick that there is **no internal**

space inside the cell. This tissue is present in stems, around vascular bundles, in the veins of leaves and in the hard covering of seeds and nuts. It provides strength to the plant parts.



6.4: Various types of simple tissues: (a) Parenchyma (i) transverse section, (ii) longitudinal section; (b) Collenchyma (i) transverse section, (ii) longitudinal section; (c) Sclerenchyma (i) transverse section, (ii) longitudinal section.

Simple Permanent Tissue

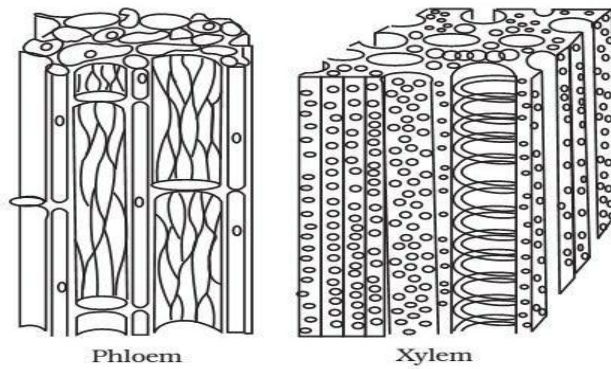
Epidermis

- What you observe is the outermost layer of cells, called epidermis. The epidermis is usually made of a single layer of cells.

- In some plants living in very dry habitats, the Epidermis may be thicker since protection against water loss is critical.
- The entire surface of a plant has this outer covering of epidermis. It protects all the parts of the plant.
- Epidermal cells on the aerial parts of the plant often secrete a waxy, water-resistant layer on their outer surface. This aids in protection against loss of water, mechanical injury and invasion by parasitic fungi.
- Since it has a **protective role** to play, cells of epidermal tissue form a continuous layer **without intercellular spaces**.
- Most epidermal cells are relatively flat. Often their outer and side walls are thicker than the inner wall.
- Small pores in the epidermis of the leaf are called **stomata**. Stomata are enclosed by two kidney-shaped cells called **guard cells**. They are necessary for exchanging gases with the atmosphere.
- **Transpiration** (loss of water in the form of water vapour) also takes place through stomata
- Epidermal cells of the roots, whose function is water absorption, commonly bear long hair-like parts that greatly increase the total absorptive surface area.
- In some plants like desert plants, epidermis has a thick waxy coating of **cutin** (chemical substance with waterproof quality) on its outer surface.
- As plants grow older, the outer protective tissue undergoes certain changes. A strip of secondary meristem replaces the epidermis of the stem. Cells on the outside are cut off from this layer. This forms the several-layer thick cork or the bark of the tree. Cells of cork are dead and compactly arranged without intercellular spaces. They also have a chemical called suberin in their walls that makes them impervious to gases and water

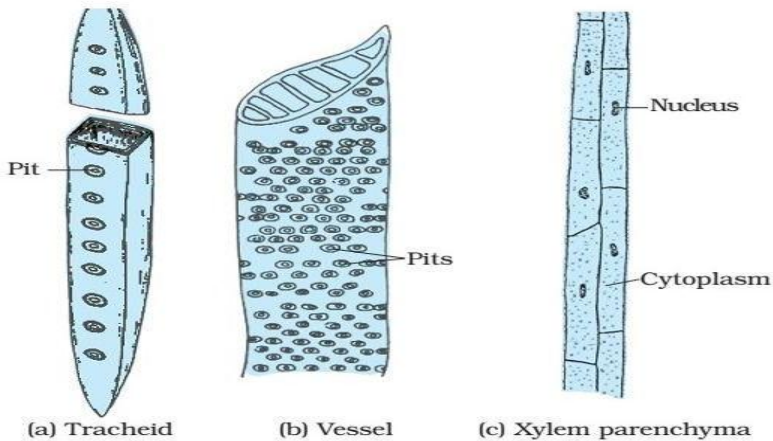
Complex Permanent Tissue

- The different types of tissues we have discussed until now are all made of **one type of cells**, which look like each other. Such tissues are called simple permanent tissue. Yet another type of permanent tissue is complex tissue.
- Complex tissues are made of **more than one type of cells**. All these cells coordinate to perform a common function.
- **Xylem** and **phloem** are examples of such complex tissues. They are both conducting tissues and constitute a vascular bundle.
- Vascular or conductive tissue is a distinctive feature of the complex plants, one that has made possible their survival in the terrestrial .



Phloem

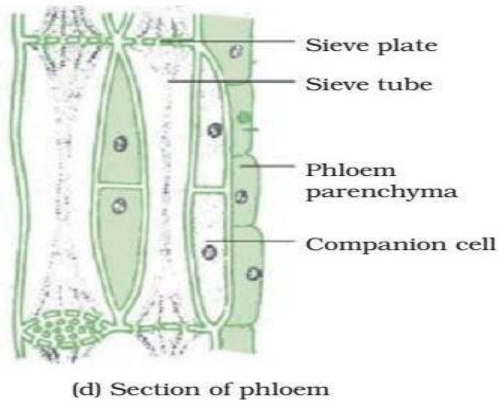
Xylem



(a) Tracheid

(b) Vessel

(c) Xylem parenchyma



(d) Section of phloem

Xylem

- Xylem consists of **tracheids, vessels, xylem parenchyma** and **xylem fibres**. The cells have thick walls, and many of them are dead cells.
- Tracheids and vessels are tubular structures. This allows them to transport water and minerals vertically.
- The parenchyma **stores food** and helps in the **sideways conduction of water**. Fibres are mainly supportive in function.

Phloem

- Phloem is made up of four types of elements: **sieve tubes, companion cells, phloem fibres** and the **phloem parenchyma**. Sieve tubes are tubular cells with perforated walls.
- Phloem is unlike xylem in that materials can move in **both directions** in it. Phloem transports **food** from leaves to other Parts of the plant. Except for phloem fibres, phloem cells are living cells.

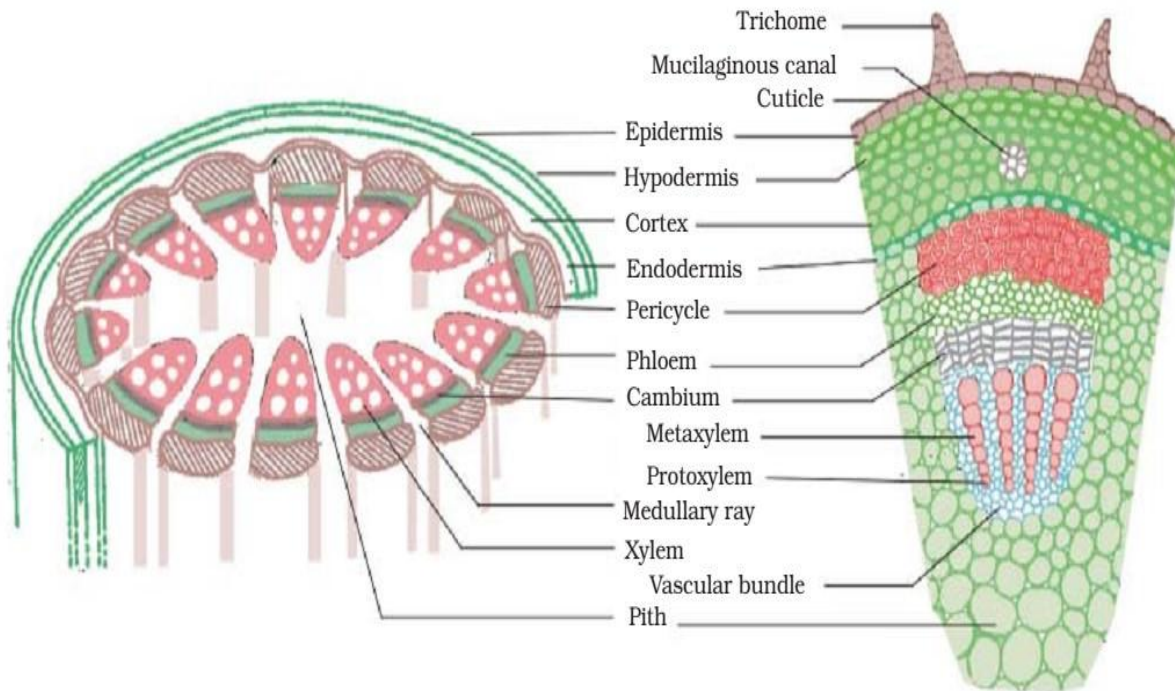


Fig. 6.3: Section of a stem

Meristem: Introduction, classification, cytological characters.

Meristematic Tissue Definition : “**Meristematic tissue is the plant tissue that has the ability to divide actively throughout its life.**”

What is Meristematic Tissue?

The term meristem was given by Carl Wilhelm von Nägeli. Meristematic tissue contains undifferentiated cells which are the building blocks of the specialized plant structures.

Meristematic tissues contain living cells with varied shapes. They possess a large nucleus devoid of the vacuole. The cells have no intercellular space. The zone where these cells exist is known as meristem.

The cells of the meristematic tissue divide actively to form specialized structures such as buds of leaves and flowers, tips of roots and shoots, etc. These **cells** help to increase the length and girth of the plant.

Characteristics of Meristematic Tissue

1. The cells of these tissues are commonly called meristems.
2. The meristematic tissue has the quality of self-renewal. Every time the cell divides, one cell remains identical to the parent cell and the others form specialized structures.
3. They have very small and few vacuoles.
4. The meristematic tissue are living and thin-walled.
5. The protoplasm of the cells is very dense.
6. The meristematic tissues heal the wounds of an injured plant.
7. The cells of the meristematic tissue are young and immature.
8. They do not store food.
9. They exhibit a very high metabolic activity.
10. They possess a single, large and prominent nucleus.

Types of Meristematic Tissue

Meristematic Tissue On the basis of Origin

Promeristem

- The earliest and youngest meristematic tissue.
- It originates from the embryo.
- The primary meristem arises from the promeristem.
- It is found in the root and the shoot tips.

Primary Meristem

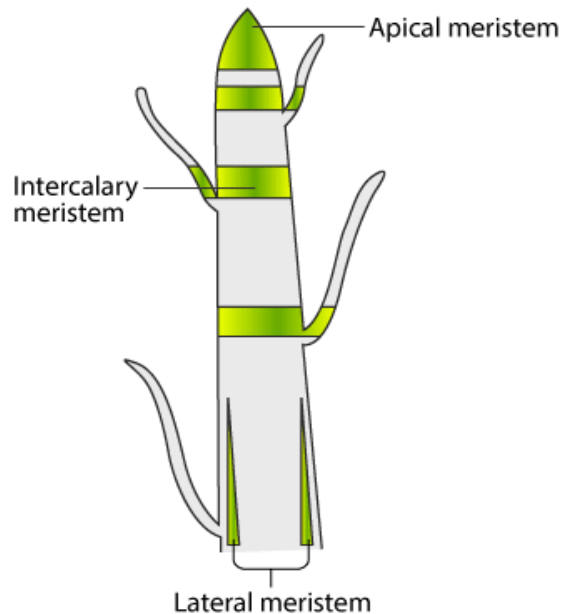
- It arises from the promeristem.
- Cells divide actively.
- It is present below the promeristem and forms the permanent tissue.

Secondary Meristem

- It originates from the primary meristem.
- The permanent tissue forms from the secondary meristem.

Meristematic Tissue On the Basis of Position

MERISTEMATIC TISSUE



Meristematic Tissue – Based on Occurrence

Apical Meristem

- These are present at the tips of the roots and shoots and help in the increase in height of the plants.
- Various cell divisions facilitate the growth of the cells in the roots and shoots. and help in cellular enlargement.
- Apical meristem is divided into-promeristem zone which contains actively dividing cells, and the meristematic zone which contains protoderm, procambium, and ground meristem.

Intercalary Meristem

- It is located in the leaves and internodes at the intercalary position.
- These help to increase the length of the internode.
- It is found in grass, monocots, and pines.
- It is a part of apical meristem and adds to the height of the plant.

Lateral Meristem

- It is located in the stems and roots on the lateral side.
- It increases the thickness of the plant.
- Vascular cambium and cork cambium are the two lateral meristems.

- These divide periclinally or radially and give rise to secondary permanent tissues.

Meristematic Tissue On the Basis of Function

Protoderm

- It is the outermost plant tissue and forms the epidermis.
- It protects the plants from any mechanical shocks.

Procambium

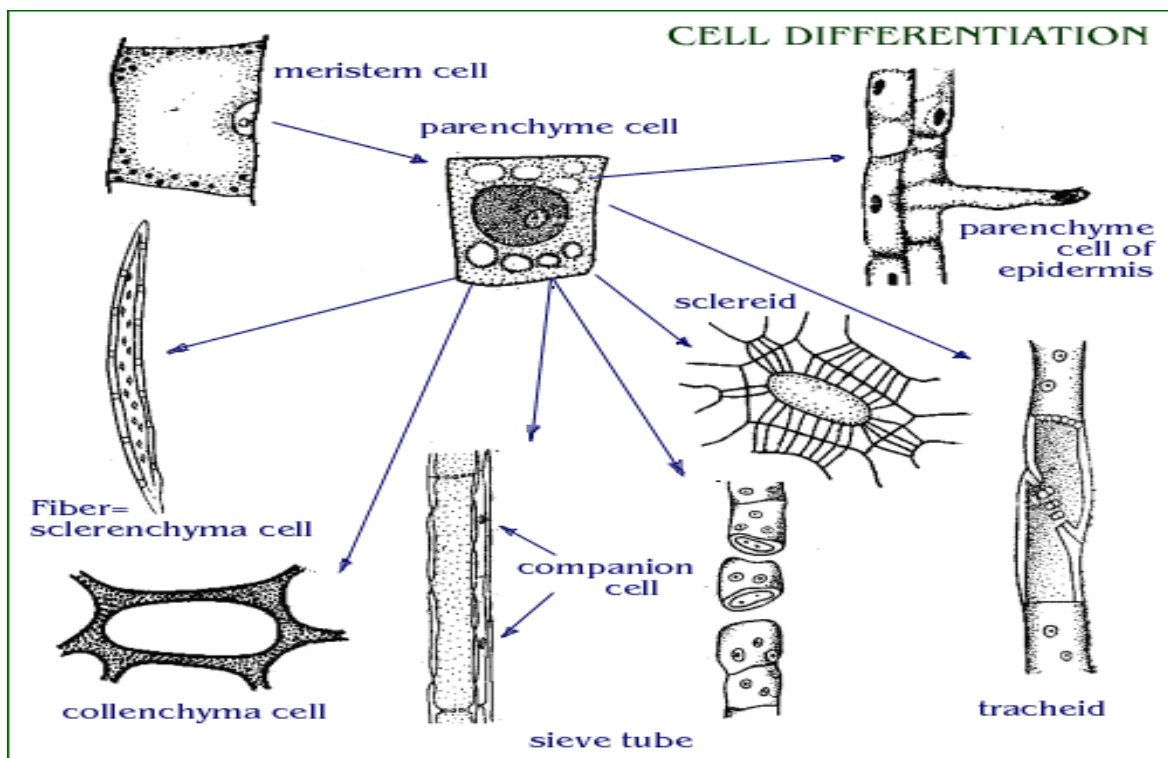
- It is the innermost tissue and gives rise to xylem and phloem.
- It helps in the transport of water and nutrients to different parts of the plant.

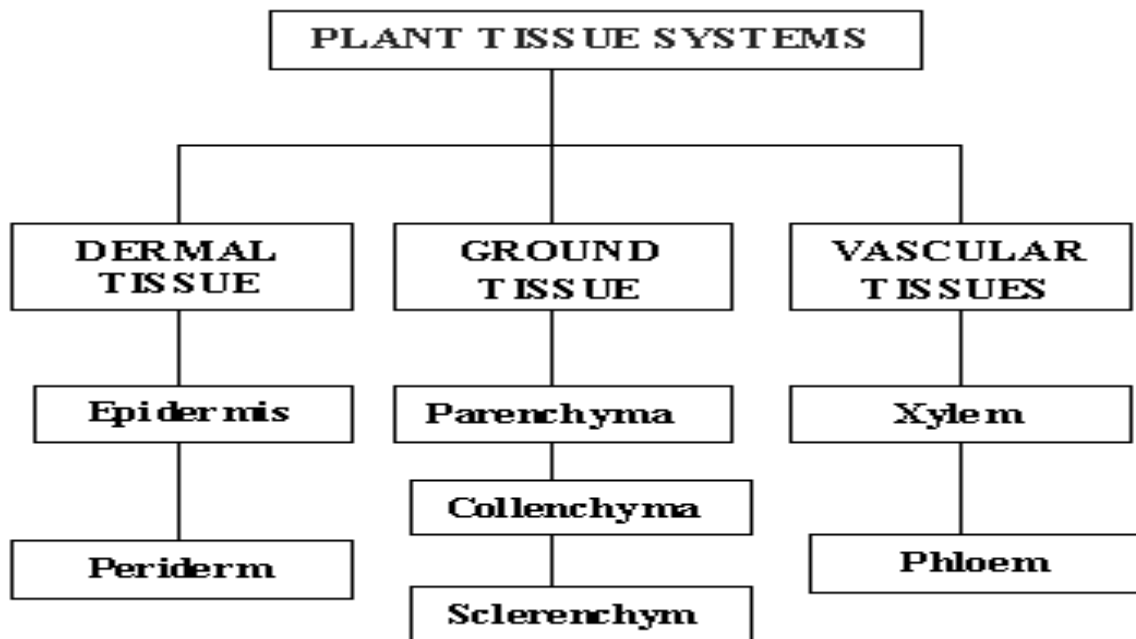
Ground Meristem

- The cells are large with thick walls.
- It forms the cortex, pericycle, and pith.

The meristematic tissue is usually found in the apices of the **root systems** and the shoots and is in a continuous state of division.

Types of cells and tissues. : There are various types of plant cells which include: **parenchyma** cells, sclerenchyma cells, collenchyma cells, xylem cells, and phloem cells. **Parenchyma** cells are the major cells of plants. They make up plant leaves and are responsible for the plants metabolism and food production.





Development of plant body: Polarity,

The characteristic orientation of organisms which is typically bipolar and axiate, is termed polarity.

Cell Polarity in Plants: PIN Proteins as **Polarity** Readouts

or **Polarity** Regulators. Auxin efflux carrier PIN proteins are the first identified cell **polarity** markers in **plants**. Based on their polar localization they guide the cell to cell transport of signalling molecule auxin.

Polarity (originates early in **development**)

The **polarity** axes are established at the stage of zygote, extending to the **developing** embryo, and they "vectorize" subsequent **plant growth** and **development**. This review deals with the **polarity** phenomena and the mechanisms of symmetry axis formation at the level of cells and **plant** tissues.

Cell Polarity in Plants : PIN Proteins as **Polarity** Readouts

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Polarity : In the development of a plant growth does not proceed at random producing a formless mass of living cells but is an orderly process that gives rise to specific three dimensional forms of organ or body. Growth in one region or dimension is related to growth in the others and thus the plant becomes an integrated individual. A notable feature of these bodily forms is the presence in them of an axis which establishes a longitudinal dimension for an organ or the plant.

Along the axis and symmetrically to it, the lateral structures develop. The two ends of poles or the axis are usually different both as to structure and physiological activity.

Thus a typical vascular plant has a major axis with the root at one end and shoot at the other, with lateral appendages, e.g., leaves, branches and lateral roots arranged symmetrically around it. Growth is usually more rapid parallel to the axis than at right angles, resulting usually, but by no means invariably, in an elongate form. These patterns appear very early in the development of an organism as the result of differences in growth or in planes of cell division. **This characteristic orientation of an organism, which is typically bipolar and axial, is termed polarity.**

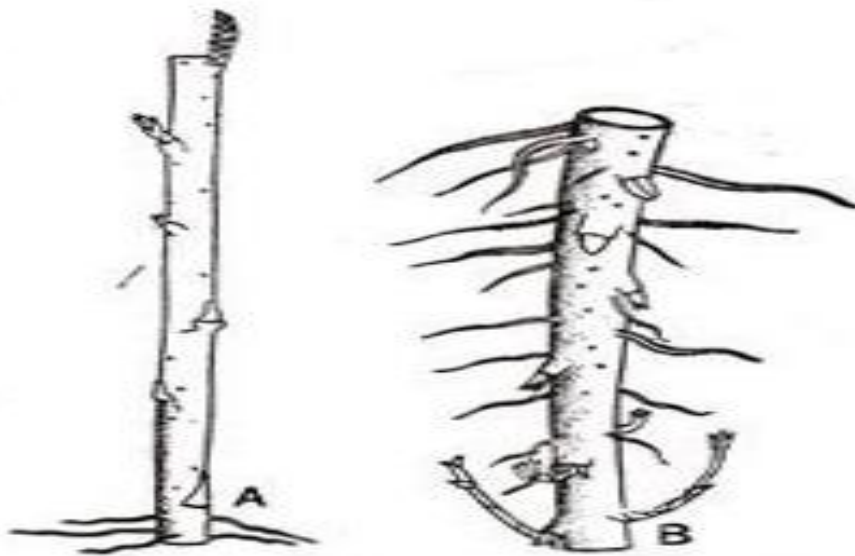


FIG. 749. Polarity in plants. Twigs of willow. A. Suspended in a moist chamber in the normal position. Note rooting from the lower end and buds near the other end, expanding into shoots. B. Twig hung in the inverted position. Note that the corresponding buds at the end which is now lowest, still give rise to shoots while roots are produced near the lower end which is now, of course, uppermost.

Polarity may manifest itself in a variety of ways. The structures at the two ends of the axis are very much unlike as in the cases of root and shoot, stem end and flowering end and petiole and leaf blade. The movement of certain substances may take place in one direction along the axis but not in the other, thus showing polarity in physiological activity. Individual Cells show polar behavior in the plane, of division and in the different characters of the two daughter cells formed.

By axiate polarity is then understood as the property of the plant or an organ which determines the contrast between apex and base. In higher plants, this polarity is already determined in the fertilized egg cell and once established it seems to be strictly followed. When a piece of stem is cut off the plant this will usually reform roots and often shoots. The roots are always produced at the basal end of the axis whereas shoots develop near the apical end.

This polarity seems to be due to polar transport of auxin in cells and tissues where it always moves from apex to base. This causes auxin accumulation at the base which favours root formation and a deficiency of auxin at the apex which favours shoot initiation. Thus it has never been possible to transform the shoot end into root end and conversely by simply inverting a plant or a piece of stem (except by treatment with morphactins).

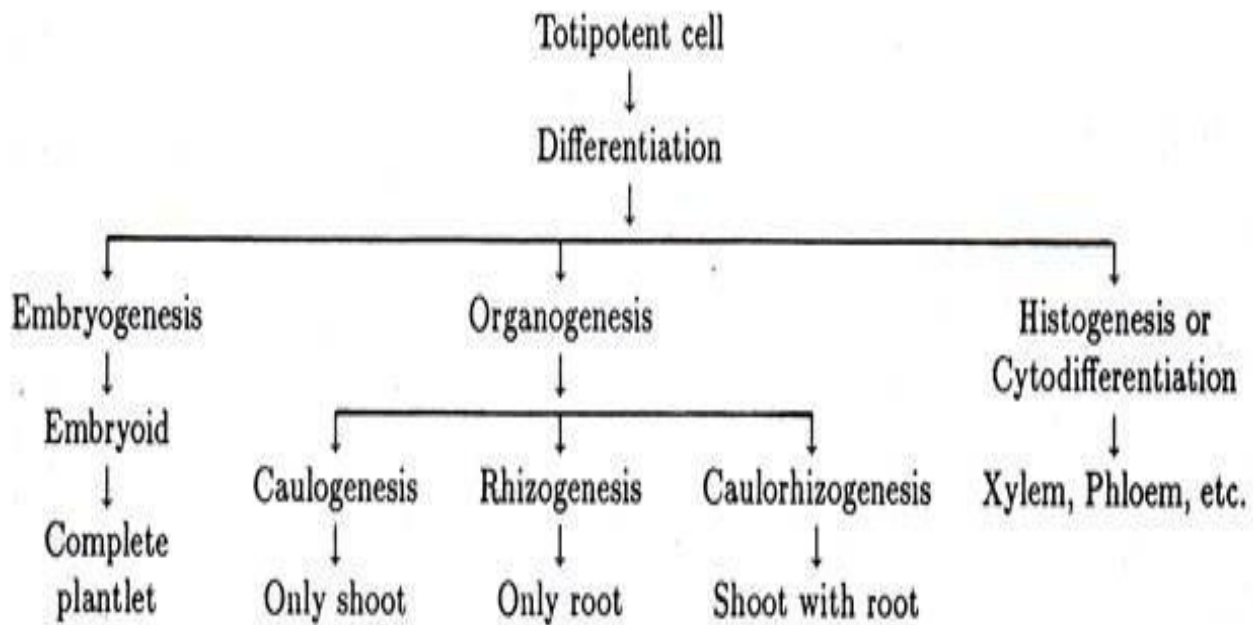
Cytodifferentiation and organogenesis during embryonic development.

Cytodifferentiation : During growth and maturation of callus tissues few dedifferentiated cells undergo cyto quiescence and cyto senescence and these phenomenon's are associated with differentiation of vascular tissues and this whole developmental process is called **cytodifferentiation**.

Dedifferentiation refers to a [cellular process](#) in which a [differentiated cell](#) loses its special [form](#) or [function](#), or reverts to an earlier developmental stage. Cell differentiation is a process in which the cell acquires modifications in form and function. As a result, the cell becomes another cell type that is specialized in carrying out a particular rather than a generalized function.

In broader perspective, **quiescence** occurs due to lack of nutrition and growth factors whereas **senescence** takes place due to aging and serious DNA damages.

Contrary to **quiescence**, **senescence** is a degenerative process ensuing a certain cell death.



It is also observed in some experiment that cells of some callus mass frequently differentiate into vascular elements such as xylem and phloem without forming any plant organs or embryoids. This process is known as “histogenesis” or “Cyto-differentiation”. Thus the totipotent cells may express themselves in different way on the basis of differentiation process and manipulation.

The following factors affecting cytodifferentiation.

(1) The chemical factors : (a) Auxin (b) Cytokinin (c) Gibberellin (d) Effect of Sugars.

(2) The physical factors : (a) Light (b) Temperature (c) Pressure and (d) Water Stress.

(1) Chemical Factors : Generally phytohormones and sugars influence greatly on Cytodifferentiation. It is evident that auxin, cytokinin and gibberellin are involved in the process of Cytodifferentiation.

a. Auxin : Auxin at low concentration stimulates xylogenesis. There is an inverse relationship between the degree of xylem differentiation and auxin concentration. The influence of auxin in xylem differentiation has been demonstrated under in vitro by many workers.

Grafting a small vegetative bud on the upper surface of the callus tissue, it has been observed that, after few days, the differentiation of vascular tissue in callus tissue takes place below the bud (Fig 6.2(a)). This observation suggests that the stimulus of vascular tissue differentiation in callus tissue is provided by the vegetative bud.

Now, if the direct contact between the bud and the callus tissue is broken by placing a semi-permeable membrane at the site of the graft, still the differentiation of vascular tissue occurs. This experiment indicates that the nature of stimulus is of diffusible chemicals. Further, a piece of callus tissue is placed on a medium which do not favour the differentiation of vascular tissue.

The callus tissue is totally undifferentiated and is free from vascular elements. Now a V-shaped incision is made on the upper surface of the callus tissue and the V-shaped cavity is filled up with 1% agar block containing auxin and sucrose. The appearance of vascular tissue is again noticed in the callus issue (Fig 6.2(b)). But in the control set the callus tissue is without any vascular tissue. Therefore, the above experimental evidence clearly indicates that auxin plays an important role in Cytodifferentiation.

b. Cytokinin:

There are some evidences to put forward that cytokinin may also be involved in Cytodifferentiation. It has been demonstrated that kinetin added medium containing coconut water enhances tracheidal differentiation in the callus tissue of Nicotianatabacum. The stimulatory effect of cytokinin in xylogenesis has also been observed in the cytoledonary callus tissue of soybean.

Nitsch and Nitsch (1960) on the other hand, have shown that storage parenchyma of Jerusalem artichoke tuber produced a natural cytokinin and cell division and Cytodifferentiation could be the outcome of a complex interaction between exogenous auxin and endogenous eye of cytokinins.

Similarly, the exogenous cytokinins in combination with an auxin do markedly increase the quantity of tracheary elements has been shown by Bergmann. But in some cases, kinetin shows some inhibitory effect on xylogenesis e.g. Coleus stem callus, callus tissue of Helianthus and Linum. Therefore, the exact role of cytokinin is not clear and remains to be determined in several forms of tissue system.

c. Gibberellin:

The influence of gibberellin in cell division and xylem differentiation has been investigated under m vitro conditions in order to established some quantitative relationship. Gibberellin interacting with auxin is effective in cell enlargement and tracheidal differentiation but not when used alone.

For cultured pea root segments in auxin-cytokinin media, the percentage of xylem differentiation is rarely up to 20 in respect to total cell population, whereas auxin-cytokinin-gibberellin treatments increase the response giving a higher percentage of differentiated cell compared to other combinations. The hormonal interactions among the three and, in particular, of the role of gibberellin in presence of auxin or auxin-cytokinin in xylogenesis, needs further exploration.

d. Effect of Sugars:

The sugar, particularly sucrose, in the medium is very essential for Cytodifferentiation. Even the effect of auxin on vascular differentiation seems to be closely dependent on the presence of sugar in the medium. Sucrose as an energy source is very important in Cytodifferentiation. At lower levels of sucrose (1.5 to 2.5%) only xylem is formed and concentration above 4% favours a balance of xylem and phloem elements. Only disaccharides which contain an α -glucosyl radical at the non-reducing end induce vascularized nodules in callus as in Phaseolus.

As a carbon source, sugar serves a dual purpose- production and deposition of cellulose and concurrently of lignification of cell i.e. deposition of lignin in the lattices of the cellulosic micro fibrils in secondary walls during Cytodifferentiation of vessels and tracheids. Fructose, mannose, xylose, rhamnose, arabinose, galactose and mannitol (2%) have not shown any positive effect in vascular differentiation as compared to sucrose.

Xylem differentiation to some extent occurs with the incorporation of cellobiose, lactose-raffinose and glucose (2%). Maltose (2%) could partially replace sucrose in xylem and phloem nodule differentiation. Glucose used alone causes the development of scattered xylem elements. Soluble starch (4%) stimulates xylem formation.

It is interesting to note that only those carbohydrates which support significant cell division also support tracheary element formation. With stem callus of *Parthenocissus vitacea* the tissue remains parenchymatous at lower concentration of sucrose (1%) in the medium, but with increase in its concentration (2.5%), the number of xylem arcs and the number of tracheary member in each are proportionally high and each arc is flanked by an internal cambium.

(2) Physical Factors:

a. Light:

In general light has proved to be inhibitory in xylogenesis although in exceptional cases as in carrot, it can be a requirement but replaceable by cytokinin. The response to light varies depending on the nature and source of the tissue, being inhibitory in some and promotive in others.

b. Temperature:

The nature of vascular differentiation is influenced by temperature conditions-whereas high temperature (35°C) proves stimulatory to xylogenesis and formation of compact wood, as in Jerusalem artichoke (Helianthus tuberosus), low temperature causes the development of undifferentiated new tissue.

c. Pressure:

Factors such as increased pressure have been shown to be stimulatory to xylem differentiation. Probably through induced ethylene production.

d. Water Stress:

Water stress is also a controlling factor in the initiation of experimentally induced xylogenesis in cultured explants of lactuca.

Organogenesis

Definition: The production of organs, either directly from an explant or from a callus culture.

- Organogenesis depend on adventitious organs arising either from a callus culture or directly from an explant or on the formation of axillary bud to regenerate whole plants from some types of tissue culture.

Organogenesis is the formation of organs, either shoots or roots. **Organogenesis** in vitro depends on the balance of auxin and cytokinins and the ability of the tissue to respond to phytohormones during culture.

In **plants**, **Organogenesis**, which is simply the process of forming new organs, occurs continuously and only stops when the **plant** dies. In the

shoot, the shoot apical meristems regularly produce new lateral organs (leaves or flowers) and lateral branches. **Organogenesis** can then occur from those cells.

Organogenesis:

Definition :

From cells of tissue culture various organs, such as, roots, stems, leaves or flowers may be initiated. This is called organogenesis. Such organ development does not require any pre-existing initials. These new organs are formed in two stages.

In the first phase (dedifferentiation) cells of the explant divide and form undifferentiated cells. In the second phase cell differentiation takes place. Organ primordia are formed from single cells or small groups of differentiated cells. These cells form small meristem with cells containing dense cytoplasm and large nuclei.

Root formation in culture is called rhizogenesis and shoot initiation is called caulogenesis.

Root formation on culture has been noted in several cases. In culture of carrot cells root formation was first observed by Nobecourt ('39b). Explants taken from any part of a plant may produce roots. Like roots shoot buds are also formed frequently. Leaves develop less frequently than roots and shoots.

Root formation on culture of Jerusalem artichoke is influenced by mineral salts, auxin, sugar, temperature and light.

Root formation stops after several subcultures. These may be due to (a) some substances required for root initiation may be exhausted, (b) culture tissue is incapable of rhizogenesis or (c) epigenetic changes of some genes may occur.

Few layers of epidermal or sub-epidermal cells from various plants under regulated condition on culture can produce organs. In Begonia rex explants from epidermal or sub-epidermal layers near the midveins of leaves can produce roots or shoots rapidly. Root initiation occurs in a medium supplemented with zeatin and NAA. Shoot initiation takes place in presence of zeatin but in absence of auxin.

In short term cultures organization of the new meristem bears a relationship with the original organization of the explant. In culture of tobacco stalk shoot primordium arises from the external phloem.

In culture of Convolvulus roots shoot primordium originates near the protoxylem. In culture of carrot cells root primordium arises in association with the protoxylem strands and when this is transferred to an agar medium, it forms a complete plant.

In long term cultures shoot and bud primordia develop exogenously. But in some cases, as in Convolvulus callus shoot primordia develop either exogenously or endogenously. Culture of explants from lower internodes produce vegetative buds, whereas explants from young upper parts or from inflorescence produce flower buds.

For flower formation high level of nitrogen, presence of cytokinin and various constituents of nucleic acid in the medium, are necessary. Presence of auxin, gibberellin and organic nitrogenous compounds have an inhibitory effect on flower formation.

Factors Influencing Organogenesis:

(1) Age of Culture :

A young culture frequently produces organs. As the culture becomes older, this capacity decreases and ultimately disappears. But there are few exceptions. The; culture of Amorphophallus retains its regeneration capacity indefinitely. Culture of carrot cells can produce roots for many years.

(2) Ploidy Level:

In culture there is instability of genome. Only a few weeks after isolation of a diploid callus various degrees of ploidy (mostly tetraploid) have been noticed in various plants (e.g. culture of medullary parenchyma of Nicotianatabacum, Haplopapptis shoots, pollens of Ginkgo).

Only in a few cases the culture tissues maintain their normal diploid sets, as in culture of tubers of Helianthus tuberosus, leaves of Crepiscapillaris and Medicago sativa.

In certain cases, as in culture of pea root callus with increase in ploidy there is decrease in organogenesis. But in some plants, under suitable conditions organs may develop with polyploid meristem. Loss of capacity to

organogenesis is reversible in some cases. This may be due to conditions of culture and other non-genetical factors.

(3) Phytohormons:

Plant hormones have some effect on organogenesis. Gautheret noted that auxin at proper concentration can induce root primordia formation in carrot explants. Skoog first said that organogenesis can be chemically controlled. Skoog and Miller observed in tobacco a high auxincytokinin ratio favours root initiation and a low ratio favours shoot initiation.

Other scientists also observed that under regulated auxin, cytokinin ratio and carbohydrate supply formation of roots and floral or vegetative buds occur. In several dicots shoot formation occurs when the ratio between exogenous cytokinin and auxin is 100: 10. But a ratio of 10: 100 favours formation of root primordia.

In monocots shoot initiation occurs on a medium with high 2-4-D and kinetin ratio for four days and then transferred to a medium lacking hormone. In absence of auxin shoot initiation occurs in some cases. For bud initiation certain plants do not require an exogenous supply of cytokinin.

Endogenous gibberellin retards root and shoot initiation. In callus during shoot initiation starch accumulates. Gibberellin lowers the concentration of starch and thereby inhibits shoot initiation.

Endogenous ethylene retards organ initiation during early stages of culture, but in later stages it helps shoot initiation. This has been observed in culture of tobacco cotyledons and Lilium bulb tissue.

According to some scientists phenolic compounds in addition to auxin are more effective for root initiation than auxin alone. On culture of Helianthus tuberosus explants in addition to the auxin the presence of sugar, light, temperature etc. play a major role in root initiation.

(4) Phosphate Concentration:

Increase in phosphate concentration favours shoot formation and suppresses or weakens root initiation.

(5) Photoperiodism and Vernalization:

Flower formation on culture can be induced by photoperiodism in Plumbagoindica and by vernalization in Cichoriumintybus and Lunariaannua.

Plantlet Formation form Tomato Leaves:

From surface sterilised young leaves rectangular explants (6 mm x 8 mm) are cut out. Each explants is placed on a tube containing 15 c.c. of Murashige and Skoog's medium, which is composed of mineral salts, thiamine (0.4 mg/l), myo-inositol (100 mg/l), sucrose (30,000 mg/l) and a growth regulator.

For root initiation 2 mg/l IAA and 2 mg/l kinetin are needed at 12 hours photoperiod and a temperature of 25°C. After initiation of root the callus is transferred to a medium containing 4 mg/l kinetin and 4 mg/l IAA. In this medium shoot "initiation occurs after four weeks. Culture is transferred to a medium without any hormone. Subculture can be done every 3-4 weeks.