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1. Anatomy in relation to Taxonomy

Study of several internal features has been found useful in solving some important taxonomic puzzles. A few are discussed here after dividing them into two broad categories:

(A) The anatomy of vegetative organs.
(B) The anatomy of floral parts and seeds.

(A) Vegetative organs:

**Trichomes:** They are sometimes remarkably uniform and may be used for taxonomic purposes. According to Inamdar (1967) the position of Nyctanthes was confirmed within family Oleaceae on the basis of structure and ontogeny of trichomes. Similarly connection between Typhaceae and Sparganiaceae could be resolved on the basis of presence or absence of sessile or glandular trichomes. On the basis of evidence from trichomes the separation of genera Ottoschulzia and Poraquilba of family Icacinaceae was established. This separation was supported by Heintzelman and Howard (1944).

**Stomata:** Kothari and Sah (1974, 75) proposed that tribes like Sophoreae, Podalyrieae and Gensteae should be considered more primitive than Dalbergieae and Phaseoleae due to presence of anomoperigenous and anisomesoperigenous stomata. Similarly, the ontogenetic studies on stomata of Caricaceae and Cucurbitaceae suggest close relationship between them due to presence of aniso-mesoperigenous and aperigenous stomata in both these families.

**Epidermis:** Metcalf reported that shape of silica bodies; warty bodies and shape of hairy structures provide good taxonomic criteria for delimiting the related taxa in many families. Similarly distribution of silica bodies among members of Cyperaceae also considered important taxonomic characters. Jain and Singh (1974) have been able to distinguish Himalayan species of Quercus (Oak) on the basis of epidermal characters.
Leaf anatomy:- Several anatomists like Metcalfe (1968), Hagerup (1953) Cutler (1965) etc. have worked considerably on leaf anatomy in relation to taxonomy.

In grasses, the general leaf anatomy has been found a very valuable character useful in grass systematic, Govindarajalu (1969) Proposed a key to distinguish various species of Cyperus on the basis of leaf anatomy.

In some plants, distributions of mechanical tissues has been found quite useful Ayensu (1974) has shown that Vellozia and Barbacenia of Velloziaceae can be distinguished from each other by the form of sclerenchyma in the leaves.

Nodal anatomy :- Study of nodal anatomy has also been found valuable for systematic. Unilalunar nodues are found characteristically among members of several families such as Anonaceae, Apocyanaceae, Solanaceae Verbenaceae, Ericaceae and Lauraceae.

Trilacurar nodes are charcetiristically found among members of Ranuneulaceae, polygonaceae and Miliaceae. Multilacurar nodes are found among members of chenopodiaceae, Ara liaceae and Degeneriaceae.

Bailey and Howard (1941) have proposed that the subfamily Icacinoideae of family Icacinaceae may be divided into two sections on the basis of nodal structure. One section having trilacunar nodes while other section having unilacunar node.

Stem anatomy :- The primary internal structures of stems appear very useful in taxonomic studies in many cases. For this purpose distribution of fibres, variations in endodermis, characteristics of pith, distribution of vascular bundles etc. have been considered. For examples on the basis of anatomical differences in pith specis of Dubantia and Fitchia may be distinguished. Variations in endodermal cells may provide systematic criterion in families like Piperaceae, Compositae and Lamiaceae.
Arrangement and structure of vascular tissues have also been found useful taxonomically in many cases. For example, Ayensu (1970) found that two morphologically similar species of Dioscorea, D. cayenesis and D. rotundata may be distinguished on the basis of arrangement of vascular bundles in the stem. Similarly, the presence of accessory cortical or medullary bundles are identifying features in some families like Nyctaginaceae, Amaranthaceae, and Chenopodiaceae. Presence of bicolлектeral vascular bundles arranged in two rings forms a characteristic feature of the stem of cucurbitaceae.

**Cambium** :- Nature and compositions of cambium have also served certain clues in dealing with phylogeny and taxonomic studies. For example, Ghouse and Yunus (1974) distinguished the species of Dalbergia of African origin from those of Indian species on the basis of cambial structure. The Indian species have multiseriate ray initials while African species have uniseriate ray initials.

**Wood Anatomy** :- Wood anatomy in relations to taxonomy has attracted wide attention and has also played an important role in solving certain taxonomic problems. A few of them are mentioned here:

1. Hutchinson (1959), Cronquist (1968) and Takhtajan (1969) considered Magnoliales as most primitive group in contrast to Engler and Prantl (1889) who considered Amentiferae as least specialized group. The characteristic anatomical features of certain members of Magnoliales such as presence of some vessel less genera, some with long but narrower vessels, oblique end walls, wood diffuse porous type, pores distributed singly and heterogenous rays support Hutchinson’s view. At the same time, Amentiferae have specialised wood such as ring porous wood, simple perforation plate, short and round vessels with opposite or alternate pitting and homogenous rays.

2. Purkayastha (1980) studied the wood anatomy of family Annonaceae and found that wood anatomy of this
family is of much advanced type than Magnoliales. Hence this study supported Hutechinson’s view of placing this family Annonaceae in a separate order. Annonales instead of keeping it in order Magnoliales.

(3) The anatomical studies made on members of parietale reveal that division of the order into parietales and Guttiferales by Wettstein is justified.

(4) The wood structure of members of family Rubiaceae supported Bremekamp’s view of dividing the family into eight sub-families.

(5) Studies of Purkayastha (1980) on calcium oxalate crystals found in wood of family sapotaceae reveal that the two morphologically indistinguishable genera – Manilkara and Mimuspos can be separated on the basis of chambered crystalliferous strands of calcium oxalate.

**Floral Anatomy:** Role of floral anatomy have also proved useful in solving some taxonomic and phylogenetic problems. Some of the important examples are cited below:

(i) Studies on floral anatomy of family solanaceaee and scrophulariaceae suggest that the two families show great affinity in the presence of anatomically Parietal placentation and in the uniformity of vasculature of the Gynoccium.

(ii) Study of the organisation of floral vasculature suggested that families like Annonaceae. Calycanthaceae and Annonaceae have originated from Ranunculaceae.

(iii) A/c to singh and and coworkers 1987 the comparative study of vascular supply of flower and and the course of placental bundles in family caryophyllaceae and Polemoniaceae suggest that the latter have originated from caryophyllaceous stock.
**Seed Anatomy:** The role of seed anatomy in solving taxonomic and phylogenetic problems is also considerable. In recent past many workers like Martin (1946), Misra (1970), Corner (1976) etc. have worked on the diagnostic anatomical features of seeds. Corner (1976) classified the bitegmic dicotyledonous seeds in two broad classes – The testal & tegmic types on the basis of differentiation of the mechanical layer. In the testal type, the mechanical layer differentiates in the outer integument while in case of tegmic types, it differentiates in the inner integument. Each type has been further divided into three sub types as exo, meso and end testal or tegmic type. Similarly Martin (1946) had also proposed a classification of seeds based on size, relation of embryo and endosperm, position of embryo etc.

Although the study of seed anatomy is still in beginning stages but such studies can prove helpful in solving problems related with adulteration of commercial and agricultural crops.

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**2. Organisation of shoot apical Meristem (SAM)**

The shoot apices represent the self-perpetuating promeristems consisting of a mass of homogeneous meristematic cells. According to a common concept, a distinct zonation is noted in that promeristem. Zonation means distinction of prominent regions differing from each other in presence of several characters such as – (i) number and nature of cells (ii) position of cells (iii) plane of cells divisions (iv) shape of cells and (v) rate of maturation of cells.

**Vegetative shoot Apex** – Shoot apex is conical or dome-shaped in outline occurring at the tips of the stem and its branches as terminal bud. In seed plants the apical meristem is a dome-shaped mass of meristematic cells. It is covered over and protected by means of young leaves formed by it. Leaf premordia are produced periodically on the flanks. The time interval between the origin of two successive leaves or whorl of leaves at the apex is designated a *Plastochron*. 
Several theories have been put forward from time to time to explain the organization of shoot apical meristem. These are –

1) **Apical cell theory** :- This theory was propounded by Hofmeister in 1857 and supported by Nageli in 1878. According to this theory a single apical cell has been found constantly present on the growing regions of many algae, bryophytes and pteridophytes governing the entire process of apical growth. However, such organization has been found only in cryptogams and not in phanerogams.

2) **Histogen theory** :- This theory was proposed by Hanstein in 1868 and supported by strasburgar. According to this theory growing apex is composed of groups of initials forming a mass of meristems of considerable depth. This mass of meristem is differentiated into three district zones, each zone is called a histogen. These are the dermatogens, the periblem and the plerome.

These three regions or histogens makeup the apical primary meristen and on further differention produce primary permanent tissues. The apical primary meristem consisting of three regions are the transitional stages between the promeristem and the primary permanent tissues.

i) **DERMATOGEN Or Protoderm** : - It is the outemost uniseriate layer. Cells of this region usually divide by radial walls and give rise to epidermis. Rarely the cells of this layer divide by tangential as well as radial walls giving rise to multiple epidermis e.g. Ficus leaf.

ii) **The Periblem** : - It is the middle region made up of isodiametric cells. The cell of this zone give rise to primary cortex by active divisions. The endodermis is also derived from the periblem.

iii) **The Plerome** : - It is a massive central or inner core. The cells of this zone divide in all directions giving rise to central cylinder i.e.
Stele consisting of medullary rays, pith, pericycle and primary vascular tissues.

Haberlandt (1914) proposed the nomenclature of protoderm for dermatogens, ground meristem for periblem and procambium for plerome.

3) The Tunica – Corpus theory :- This theory was proposed by Schmidt (1924) and supported by Foster (1949). According to this theory the shoot apex has two tissue regions viz. (i) Tunica & (ii) Corpus.

i) The tunica : - It is the outer mantle like layer consisting of comparatively smaller cells. The cells of this region divide by anticlinal divisions and therefore, take part in surface growth only. Cells derived from tunica give rise to epidermis. If the tunica is made up of more than one layer in thickness, the outer layer differentiates into epidermis while the inner layer forms the cortical tissue in stem and interior tissue of leaf.

ii) The Corpus :- It is the central massive region surrounded externally by tunica layers. The cells of this region are larger and divide in all planes viz. anticlinal, Periclinal, tangential & radial. The dervatines of corpus result in the Pith, the vascular region and part of cortex.
Gradual differentiation or Zonation of shoot apex meristems:

a) **Shoot-Apex of Angiosperms**: Apical cell theory does not hold good as apices of spermatophytes are composed of more than one cell and hence can not be interpreted in the light of this theory.

According to histogen theory, the shoot apex of angiosperms consists of three histogenic layers such as (i) dermatogens (ii) periblem and (iii) Plerome which are laid out from the apical meristem or promeristem. But in many plants distinction and derivation of these regions are not very distinct and have no morphological significance.

In the early decades of twentieth century, the histogen theory was replaced by the tunica – corpus theory. According to this theory the shoot apex consists of two zones of meristematic tissues – a central core called corpus enveloped by one or more outer layers called tunica. Tunica and corpus, though not always sharply distinct, are independent and self perpetuating meristems. It has been found that the number of layers is not always constant in a particular genus or family or even in a species. Further it has been found that the number of layers in tunica varies in a given plant even during different stages of development of the vegetative apex.

According to Eames “Tunica and corpus should be recognized as dynamic and fluid not functionally or morphologically distinct or constant regions.

According to Popham (1952) the terms tunica and corus should be replaced by Mantle and core respectively.

The Mantle is composed of all the distinct peripheral layers of the apex in which cells divide anticlinally while the core is composed of the remaining central tissues in which cells divide in various planes. Popham (1952) on the basis of internal
organization has recognised two main types of corpus in angiosperm shoot apex such as (a) the usual angiosperm type and (b) the opuntia type.

a) **Angiosperm type** : - In this type three zones can be distinguished in the corpus –

i) The zone of central mother cell – This zone is located below the tunica initials and probably represents the corpus initials.

ii) The rib meristem.

iii) The flank meristems

b) **Opuntica type** : - In addition to the above three zone, a cambium like transitional zone can be distinguished which is cup-shaped and is situated between central mother cells and the rib and flank meristem.

Eames (1961) has suggested two types of angiospermic apical shoot organization. One in which there is no periclinal divisions in tunica cells while in other cells of tunica divide anticlinally except some central cells of the apex where periclinal divisions occur. According to him, “evolutionary progress in the zonation of the shoot apex has been from a tunica with layers in which periclinal divisions restricted to the apex and to those with anticlinal divisions only”.

Newman (1965) has classified shoot apices in three main types on the basis of a group of permanent initials cells occurring in the shoot apex –

1) **Monoplex type** : - In this type meristem is superficially situated which is pointed inwardly. E.g. Fern and other related genera.

2) **Simplex type** : - This type of shoot apex is characterized by parallel sided meristematic residue restricted to superficial layer. E.g. Gymnosperms.
3) **Duplex type** : - Here the meristmatic residue is parallel – sided but is divided in two layers – Superficial layer which divides anticlinally and inner most layer(s) which divides both anticlinally and periclinaly. E.g. Angiosperms.

![Diagram of leaf structure](image)

L.S THROUGH STEM APEX OF VINCA SPECIES SHOWING DIFFRENTIAATION OF TUNICA AND CORPUS
3. Root Apex (Root Apical Meristem)

Due to the greater simplicity of the root apex and better distinction of histogenic zones, the anatomy of the root apex was well known long before the critical studies of shoot apex were made. It is simple and shorter than that of the shoot. The root apex differs from the shoot apex in lacking developing appendages and segregation into nodal and internodal regions in having clearer and more constant zonation. But is complicated at the tip by the root cap and different methods of formation of this structure. The root cap is a terminal portion of the root-tip covering and protecting the initiating apex of the root proper. It is formed by the same initial as those which build the root proper except monocotyledon where it has independent origin.

Growth proceeds in cap and root proper in opposite directions. In root cap the sequence of development toward the tip while away from the tip in the root itself. The initials vary from one to many. Where the initials are more than one, they are arranged in one to four fairly distinct, uniseriate groups. In each group there are one to several initials. Furthermore, the number of initials in group seems to vary with diameter and rapidity of growth of the root. In slender roots as in some grasses, it may be reduced to one but the zonation remains clear. In case where there is more than one group, the groups lie adjacent to one another on the longitudinal axis of the root.

Each of these groups quickly develops one or more growth zones which are clearly marked. Organisation of root apical meristem have long continued under the histogen theory as in may plants these zones appear to represent the “histogen”.

Hanstein (1868) put forth histogen theory proposing that in root apex regions, three distinct histogens are found. Although the term
dermatogens, periblem and plerome are no longer in general use. Haberlandt (1914) proposed the terms protoderm, ground meristem and procambium respectively for these histogens. A fourth histogen, “Calyptrogen” differentiates where the cap has an independent origin.

Tunica and corpus are terms not applicable to the root apex because of its markedly different morphology.

**Concept of quiscent zone :** - Clowes 1916 observed a central cup-shaped zone in the root tip of Zea mays. The cells of this zone have fewer mitochondria and ER, very small nuclei and low rate of DNA, RNA and protein synthesis. He called this inactive region as quiescent centre since then such regions have been observed in many plants.; Cells of this region, though inactive but many become active when active initials are damaged. Thus, this region acts as reservoir of cells. These undividing cells offer more resistance to damages and irradiations than actively dividing cells.

**Root Apex in Angiosperms :-** The root apex meristematic layers of angiosperms are usually formed by three or four groups of initiating cells.

In dicotyledons, the distal group forms the cap and the dermatogens, the median group forms the periblem and the innermost forms the plerome. In monocotyledons, the distal group forms independently the cap, the median forms the dermatogens and periblem and the innermost the plerome. The common origin of cap and dermatogens in dicotyledonous root apex shows a resemblance to the primitive type of root apex origin where both cap and epidermis are formed by a solitary apical cell (Guttenberg, 1947).

According to Guttenberg (1940, 47, ’60) in the root apex, the meristems of different tissue systems can be traced at various distances from the central cells i.e. permanent initials. In Some species the
Temporary initials of various tissue systems become distinct immediately adjacent to the central cells. This is called closed type. Such initials represent the initials of the cortex, the vascular cylinder and initials of protoderm and root cap. In other species, the meristems of different tissue systems finally become distinct only some distance away from the central cells. This is called open type. In this type, common initials either for the meristems of all the tissue systems or for the root cap, cortex meristem, and protoderm appear on the periphery of the central cells.

There is another theory called Korper – Kappe theory proposed by Schuepp (1917) and supported by Clowes (1961). According to this theory, the cells at root apex divide in two planes. The zone with inverted ‘T’ type of division was referred to as Korper (cap) whereas the other with straight ‘T’ as Kappe (body).

Though Korper type of T – division has been noted among the members of the family Gramineae and Fagaceae but it fails to explain the differentiation of cells in different species.

**Root Apex of Gymnosperms** :- In Gymnosperms, two groups of initials are found at the root apex, Inner group forms the pleurome and the outer group forms the cap and the periblem. There is no sharp demarcations between these two regions. Dermatogen, though not demarcated at the apex or tip but is originated from the periblem a little below the apex where the base of the root-cap is separated from the periblem as a “distal proliferations”.

**Root Apex of Pteridophytes** :- Either Single apical cell or a few apical initials are present in root-apex of pteridophytes. Where there is single apical cell, it is tetrahedral and it divides in such a way that new cells are added to the body of the root from its upper surface and to the root cap from its base. In Equisetum spp., ophioglossaceae etc.
the entire root develops from a single apical cell while in Marattiaceae it develops from a few apical initials.

L.S. OF ROOT APEX SHOWING DIFFERENT HISTOGEN LAYERS
4. Differentiation of Epidermis with special reference to stomata or Epidermal tissue system

**Tissue system**: - Group of one or more tissues which is structurally and functionally organized into a unit performing the common physiological function regardless of its position or continuity in the plant-body is known as tissue system.

Sachs (1875) recognized three basic tissue systems found in the primary structure of plants:

i) Epidermal tissue system

ii) Ground or fundamental tissue system

iii) Vascular tissue system.

These tissue systems originate from meristems which are outlined as follows:

**Epidermal Tissue System**: - This tissue system is formed by epidermis with its associated structures. The epidermis constitutes a layer on the entire outer surface of the plant body. It forms a continuous
layer or layers except interrupted by stomata or lenticular openings. Typically, it consists of a single layer of cells, in a few plants it is biseriate or multiseriate. Multiple epidermis is found in the leaves of Ficus sp. (Moraceae), Nerium Sp. (Apocynaceae) members of piperaceae, etc.

According to DeBary (1877) the cells of the inner most layer of the multiple epidermis of leaves usually function as a water-storing tissue.

Structure of epidermal cells:

Cell contents: The epidermal cells living, possessing large central vacuole and the peripheral uninucleate cytoplasm. Minute leucoplasts are present but chloroplasts are absent except in the guard cells of stomata and in plants of aquatic or moist and deeply shaded habitats. Mucilage, tannin and crystals may be found occasionally.

Morphology: The cells vary much in size and out-line but are essentially tubular. They are compactly fitted without any intercellular spaces. In surface view they appear more or less isodiametric or elongated in shape. In the leaves and petals of flowers these are of irregular shape. In general, elongated epidermal cells are found in stems, petioles, veins of leaves, etc. They are often lobed & toothed especially in petals or leaves.

Wall structure: The epidermal cells often have unevenly thickened walls, the outer and the radial walls being much thicker than the inner wall. This additional thickness and the cutinization of the walls is of much importance for mechanical protection and prevention of waterloss cuticle is present all over the body except in roots and some submerged aquatic plants. The epidermis of root without cuticle is called epiblema.
Ontogeny and Duration of the Epidermis: - The epidermis develops very early as a superficial layer in the apical meristem. In stem this is perhaps the first zone to become distinct when a distinct tunica is present the epidermis is formed by anticlinal divisions of the outer layer of tunica. Where tunica and corpus are not distinct, the epidermis is formed by anticlinal and periclinal division of the dermatogens. In root the epidermis is formed from the root apical meristem covered by root cap.

In perennial stems epidermal cells live until the development of a periderm layer. In leaves, flowers and most fruits epidermal cells normally live as long as the organ of which they are a part. In roots the epidermal cells become life less and lignified or suberized after the root hairs cease to function.

Function of the Epidermis: - Epidermis is mainly protective tissue. It protects the internal tissues against mechanical injuries and checks excessive loss of water during transpiration. It may also serve secondarily in photosynthesis, secretion, water storage and water absorption (epiblema of root).

The Stoma: - Stomata occur on most of the green aerial parts specially on leaves and young stems. Roots lack stomata. A stoma consists of a pore bounded by two specialized epidermal cells called guard cells. The guard cells along with the stomatal aperture constitute the stoma proper. The adjoining epidermal cells often differ morphologically from the rest of the typical epidermal cells. Such cells are called subsidiary or accessory cells. The subsidiary cells may or may not be ontogenetically closely related to the guard cells.

In most dicots, the guard cells are semilunar which are attached to each other by the curved ends of their concave sides leaving a slit-like opening. In many monocots the guard cells are dumb bell-shaped having a thick wall and narrow pore. Their wall is unevenly thickenes. The wall surrounding the pore is thickened and in elastic but the remaining part of the wall remains thin, elastic and permeable. The radial walls have characteristic fan-like micellae, composed of cellulose. Each guard cell has a cytoplasmic lining and a large central vacuole filled with cell sap. The cytoplasm contains a nucleus and a
number of chloroplasts. Electron microscopic studies have revealed that this chloroplast has fewer and less well-organised lamellae than those of mesophyll cells.

Just beneath each stoma, a prominent cavity is found called substomatal chamber. Through this chamber gaseous exchange occurs by stomatal pore.

**Distribution of stomata** :- The number of stomata in a definite area of leaf varies from plant to plant. Stomata occur abundantly on leaves. Five categories of stomatal distribution have been recognized in plants. These are :

(a) **Apple or mulberry type** :- Stomata on only lower surface of leaf.

(b) **Potato type** :- Stomata are found on both surfaces of leaf but more on lower surface than those on upper surface e.g Bean, Tomato.

(c) **Oat type** :- Stomata are found distributed equally on both surfaces of leaf e.g. Maize, Oat etc.

(d) **Wild lily type** :- Stomata are found distributed only on the upper surface of leaf e.g. lotus.

(e) **Potamogeton type** :- Stomata are altogether absent or vestigial as in submerged hydrophytes.

According to metcalfe and chalk (1950) the dicotyledons show four principal stomatal types with regard to the neighbouring epidermal cells :-

(1) **Anomocytic type** :- No subsidiary cells are present. Several ordinary epidermal cells irregularly surround the stoma. It is also called irregular celled type. e.g. in Ranunculaceae, Papavaceae; capparidaceae etc.

(2) **Anisocytic type** :- There are three subsidiary cells, one distinctly smaller than other two. It is also called unequal celled type. e.g. In members of cruciferae, Solanaceae, convolvulaceae etc.
(3) **Paracytic or Paralled celled type** :- In this type one or more subsidiary cells flank the stoma on either side which lie parallel to the long axis of the guard cells. e.g. Rubiaceae, Magnoliaceae etc.

(4) **Diacytic type** :- In this type stoma remains enclosed by a pair of subsidiary cells with their common wall at right angle to the long axis of the guard cells as seen in members of Caryophyllaceae, Labiataeae etc.

**Position of stomata** :- Stomata may occupy three different positions with regard to epidermal cells –

(a) Stomata found at the same level as in most mesophytes.

(b) Stomata found in depression and are called sunken stomata as in xerophyts.

(c) Stomata are slightly raised above the surface of epidermis as in solanum, prunus etc.

**Development of Stomata** :- In Gymnosperms the stomata are commonly deeply sunken. Florin and his co-workers recognized two types of stomata based on ontogeny :-

(a) **Haplocheilic type** :- In this type guard cells arise by single division of stomatal initial and subsidiary cells are not related ontogenetically to the guard cells e.g. Gnetum, welwitschia etc.

(b) **Syndetocheilic type** :- A protodermal cell divides into a guard mother cell and two lateral cells. Each of the lateral cells thus formed either becomes subsidiary cell or give rise to subsidiary cells by further division.

**Development of stomata in Angiosperm** :- The stomata originate from the protoderm Protodermal cell divides unequally into two cells. Of these, the smaller cell acts as the precursor or mother cell of the guard cell. It divides into two cells. These resultant cells take the characteristic shape of guard cell through differential expansion. The intercellular substance between the cells is weakened. They separate in the median parts and the stomatal opening is thus formed. Development
of subsidiary cells take place from protoderm cells lying close to stomata mother cell.

Various spatial readjustments occur between the guard cells and the adjacent subsidiary or other epidermal cells. Due to this spatial adjustment the guard cells may be elevated above or lowered below the surface of the epidermis. Finally the adjacent cells may overarch the guard cells or grow under them into the substomatal chamber.

In monocotyledones the formation of stomata begins at the apices of the leaves and progresses in downward direction while in dicotyledons the different developmental stages are mixed in a mosaic fashion.

Pant classified stomata into the following types on the basis of ontogeny:

1. **Mesogenous**: In this type, both subsidiary cell and guard cell develop from the same meristemoid.

2. **Perigenous**: Here the subsidiary cells are formed by cells lying around the meristemoid that divides to form the guard cells.

3. **Mesoperigenous**: In this type, surrounding cells are dual in origins e.g. Ranunculaceae.

**Functions of stomata**: Stomata are very important structures from physiology-point of view. As interchange of gases takes place between the outer atmosphere and intercellular spaces of the internal tissues through them, hence physiological functions like photosynthesis, Respiration and transpiration are facilitated by stomata.

**Trichomes**: They are highly variable appendages of the epidermis. They occur on all parts of the plant and may persist through the life of a plant part or may fall off early. Sometimes they are remarkably constant and so characteristic that they may be used for taxonomic purposes. Trichomes include glandular or nonglandular hairs, scales, papillae, absorbing root hairs etc.
There are other structures such as warts, spines etc. which consist of epidermal as well as subepidermal tissues. They are called emergences.

Epidermis in surface view illustrating formed by guard cells and surrounding cells.
Epidermis of a grass – sugar cane (saccharum) – in surface view. A, lower epidermis of leaf with stomata. B, epidermis of stem with cork cells and silica cells

Development of stomata. Guaer-cell precursors (mother cells) have been formed by a division of a protodermal cell (A, D). The precursor has divided into two guard cells (B, E). Stomatal Opening has been formed (C, F)
Trichomes A, simpale hair form Cistus leaf. Structure resembling a short hair is included at base. B, Uniseriate hair form Saintpaulia leaf. C, D, tufted hair from leaf of cotton (Gossypium). E, Stellate hair from leaf of alkali mallow (Sida). F, dendroid hair from lavender leaf (Lavandula). G, short multicellular hair from leaf of potato (Solanum). H, I, peltate scale from leaf of olive (Olea). J, biocellular hair from stem of pekagonium,
5. Nodal Anatomy

Nodes and internodes may be well demarcated or may be indistinct. Anatomically a node may be defined as a region or place from where leaf traces or branch traces arise. The vascular cylinder shows different patterns in the region of nodes and internodes.

Leaf traces and leaf gaps: The vascular connections of leaf and stem is seen in the nodal region where one or more strands in the stem bend away from the stem toward to leaf. The vascular strands of leaves and stem are connected because the stem and leaves are structurally continuous. The bundle extending from the base of the leaf to the point of its junctions with another strand in the stem is called leaf trace. Thus, the leaf trace may be defined as the cauline part of vascular supply of leaf. The foliar part of this supply begins at the base of the petiole and extends into the blade. A leaf trace may also be defined as a vascular bundle that connects the vascular bundle of the leaf with that of the stem.

In the nodal region, where a leaf trace is bent away from the centre of the stem towards the leaf base, a region of parenchyma occurs in the vascular cylinder of the stem. This region, which appears in T.S. like a wide interfascicular regions is known as leaf gap. A leaf gap thus is a parenchymatous region is the vascular cylinder of the stem located opposite the upper part of a leaf trace.

Structurally, leaf traces are strands of primary vascular tissue. The proximal part of leaf trace consists of xylem alone but the distal part of the trace is made up of both xylem and phloem.

The traces supplying a leaf range in number from one to many and the number is usually constant for a given species and often for a family. Hence this is of taxonomic and phylogenetic importance.

Relationship of leaf trace with nodes: There are three common types of nodes found in dicotyledons:
i) Unilacunar with single trace: In this type there is one leaf gap with single strand of leaf supply. In unilacunar double traces, there are two vascular strands entering the leaf at the node with single gap.

ii) Trilacunar: A node with three gaps and three traces to a leaf (one median and two laterals).

iii) Multilacunar: A node with several gaps and traces associated with a single leaf.

In most monocotyledons, leaves have sheathing leaf bases which receive a large number of leaf traces separately inserted around the stem. In Gymnosperms, unilacunar node is common. In ferns, variable number of leaf traces enter in a leaf but they are always associated with a single gap.

**Branch traces**: The vascular bundles that connect the main stem with the branch may be recognized in the nodal region. These strands are called branch traces or ramular traces. Branch traces are usually in the form of two bundles, less often one bundle. Actually branch traces are also leaf traces i.e. leaf traces of the first leaves called prophylls of the branch. In conifers and the dicotyledons two prophylls occur opposite one another. Because of this arrangement, two traces connect the bud with the main axis. A branch trace is located above the leaf trace.

**Branch gap**: A branch trace is also associated with the formation of a break or interruption in the vascular cylinder around and above the point of departure of the trace. The opening or break through which continuity of the pith and cortex is established is called branch gap. Branch gaps are larger than leaf gaps and are more extended is the axis. Branch gaps are found in all siphonostelic plants (Stele with pith) and not found in protostellic plants (Stele without pith).

**Evolutions of nodal structure**: Sinnot (1914) proposed that trilacunar condition is the most primitive is nature from which unilacunar and
multilacunar condition arose by reduction and amplification process respectively. His proposition was widely accepted.

However, Ozenda (1949) proposed that three nodal types form a regressive series i.e. multilacunar – trilacunar – unilacunar.

Canright (1955) and Bailey (1956) proposed that unilacunar with two distinct traces is the primitive type among angiosperms. It was supported by many other anatomists as this type of node is characteristic not only of Gymnosperms and ferns but also occur in several members of dicot families such as Labiatae, Solanaceae and Verbenaceae.

Takhatajan (1964) also proposed that trilacunar node with two traces from the central gap is the basic type from which arose other types of nodal structure either by reductions or by multiplication.
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PG SEMESTER – II, PAPER – VII

BOT523: Plant Anatomy and Development Reproduction and Plant resources Utilisation and conservation
1. Classification of endosperm and its development.

INTRODUCTION

- Endosperm is a nutritive tissue found in the seed plants, namely, gymnosperm and angiosperm
- In gymnosperms, the haploid nucleus of the functional mega pore divides repeatedly without cell wall formation and gives rise to a free nuclear structure, known as endosperm or female gametophyte, whose cells are haploid.
- On the other hand, in angiosperms, the functional megas pore inside the ovule develops into a sac – like structure containing eight nuclei, designated as female gametophyte or embryo sac.
- Out of eight nuclei, two hyploid nuclei at the centre of the sac are known as polar nuclei, three at the micropylar end form the egg apparatus, three at the chalazal end constitute the antipodal cells.
- After pollination, pollen grains on the stigma of the pistil germinate and the germtube proceeds towards the micropyle of the female gametophyte containing two male gamemates at the tip.
- The pollen tube enters the embryo sac through the micropyle and releases the male gametes
- One of the male gamete fuses with the egg cell forming emlyro whereas the other fuses with two polar nuclei giving rise to the endosperm which is triploid

- Hence, endosperm is a triploid structure present inside the ovule and acts as main source of nutrition for developing embryo. However, endosperm formation is suppressed in two angiosphermonic families the orchidaceae and podostemonaceae
TYPES OF ENDOSPERM

On the basis of the mode of development, endosperm is categorised into three types:

(i) Nuclear endosperm
(ii) Cellular endosperm
(iii) Helobial endosperm

NUCLEAR ENDOSPERM:

- In this type of endosperm formation, the first division and several subsequent divisions of the primary endosperm nucleus are not accompanied by wall formation.
- The nuclei either remain free in the embryo sac or in later stages wall formation may occur partly or completely.
- Usually first few divisions of the primary endosperm nucleus (PEN) are synchronous, but in later stages the synchrony is disturbed.
The nuclei are found at different stages of division some in metaphase, some others in anaphase, telophase or still prophase.

Thus the number of nuclei may not always be a multiple of two.

With the progress of the divisions the nuclei are gradually pushed towards the periphery of the embryo sac.

The nuclei usually aggregate at the micropylar and chalazal ends leaving only a few on the lateral sides.

As a result, the centre of the embryo sac is occupied by a large central vacuole.

Frequently, the nuclei at the chalazal end are larger than those at the micropylar end, e.g. Ranunculus (Schurhoff, 1915).

The number of free nuclear division varies from species to species.

Wall formation, if occurs, is centripetal or basipetal.

That is, from periphery towards the centre or from micropylar end towards chalazal end.

Less frequently, cell plates are laid down simultaneously throughout the whole sac. e.g. Tacca (Betow, 1931) and Carya (McKay, 1947).

Whatever is the mode of wall formation, eventually the entire sac is filled with cells, or there are only one or two layers of cells at periphery, or only at the micropylar end e.g., crotalaria. However, in the family Caryophyllaceae all the three conditions are found in different genera (Rocen 1927).

The number of nuclei in the endosperm cells is not fixed. Presence of one, two or more nuclei is very commonly found which either remain free or may become fused resulting into difference in the ploidy of each cell exceeding tripliod condition.
- Sometimes, single nucleus in a cell may also divide resulting into a multinucleate cell.

- An interesting condition is reported in Musa errans (Juliano and Alcala 1933.)

- In this species, some of the endosperm nuclei divide more actively and become sheathed by a common cytoplasmic wall forming a number of “nodule” like structures in the embryo sac called Endosperm Masses.
Special mention must be made here that in most of the members of protiaceae, the micropylar half of the endosperm becomes cellular, but the chalazal half remains free nuclear and forms a vermiciform appendage, named as haustoria. (Kausik, 1938)
Nuclear endosperm in cocos nucifera (coconut) also deserves special mention. The peripheral cellular part of the endosperm is the edible coconut meat and the coenocytic part is the liquid coconut milk which is a well known growth regulator for plants and is used as a healthy drink by mankind. The fruit of Areca Catchue (Betel nut) has also the same endosperm structure, only difference is the small size of the cavity.

**CELLULAR ENDOSPERM**

- The cellular endosperm is characterized by first nuclear division immediately followed by a transverse wall.
- Sometimes vertical or oblique wall formation also occur.
- However, in senecio (Afzelius, 1924) the orientation of the wall is not constant.

- Anoxia, moschatellina (lagerberg, 1909) is well known example, in which first and second division are always vertical resulting into four cylindrical cells. The third division is transverse.
and eight cells are formed arranged into two tiers. Next division is also transverse, but further divisions are irregular.

- Like nuclear endosperm, occurrence of haustoria in the cellular endosperm is also very common. They may arise either at the micropylar end or at the chalazal end, or at both ends in the same genus.
- A very well developed, large and aggressive micropylar haustarium is reported in Impatiens roylei (Dahlgren, 1934)
- First division of the PEN is transverse and results into two unequal chambers.
- The micropylar chamber is smaller and divides transversely into three chambers.
- The uppermost chamber contains the zygote and forms a giant branched haustorium. The branches are so large that they extend upto funiculus.
- The next chamber gives rise to a group of cells which lie in close proximity to the young embryo.
- The third and lowermost chamber in which nuclear divisions are not followed by wall formation, fuses with the chalaza and the whole chalaza part remains free nuclear. However, in later stages wall formation takes places.
- The chalazal haustorium is reported in magnolia obovata
- First division of the PEN is transverse resulting into two equal chambers.
- Divisions in the micropylar chamber are rapid and a large cellular micropylar endosperm is formed in which the embryo remains embedded.
- The chalazal chamber divides transversely at a slow rate and a long 2-called tail like haustorium is formed

![Diagram of embryo and endosperm](image)

*Fig. Chalazal Haustorium in Magnolia obovata*

- In Malampyrum linear and nemophila insignis, both micropylar and chalazal haustoria are seen.
- In melampyrum the micropylar haustorium is 4- nucleate with many tubular processes, one of which extends up to funiculus.

- The chalazal haustorium is two nucleate and is broad above gradually tapering below. The cellular endosperm remains in the centre.
• A unique condition is observed in the family Loranthaceae (Treub, 1885; Johri and Maheshwari, 1950).

• In the genera of this family ovary lacks ovule. The sporoginous tissue located at the base of the ovary develops several embryo sacs which elongate considerably, some of them even enter the style. After double fertilization, the PEN of each embryo sac moves to the basal part where it divides to form a cellular endosperm. During further development, the endosperms of all the embryo sacs in an ovary enlarge and fuse to produce a composite endosperm mass.

• Several proembryos belonging to individual embryo sacs with long suspensors develop but only one survives and attains maturity.
HELOBIAL ENDOSPERM

- This type of endosperm is intermediate between nuclear and cellular types. The PEN moves to the chalaza end of the embryo sac and divides there followed by wall formation which results in the partition of the embryo sac into two unequal chambers.
- The micropylar chamber is much larger than the chalazal.
- In the micropylar chamber several free nuclear division take place whereas in the chalazal only a few divisions take place.
- The nuclear division in the micropylar chamber is more rapid than chalazal chamber.
- As a result, the former has large number of nuclei and latter has only a few.
- In later stage, wall formation occurs, mostly in the chalazal chamber.
- But in most of the cases this chamber is crushed with the development of embryo.
• This type is usually found in the order helobiales and many monocotyledonous plants.

Like nuclear and cellular endosperm, the helobial endosperm also have haustoria.
• The haustoria are remarkable in being lateral in position e.g. - monochoria.

MARPHOLOGICAL NATURE OF THE ENDOSPERM

• It is now well established fact that the endosperm is a formless mass of tissue with negligible or no differentiation. If any, it has very
short life – span. It is either consumed during seed development or when the seed attains maturity.

- The variation in the number and composition of the chromosome in the nuclei of the endosperm has resulted in a number of speculations about its morphological nature.
  - (1) Hofmeister (1858) stated that the endosperm is a gametophytic tissue and its growth is postponed till the entry of the pollen tube in the embryo sac.
  - This opinion was later on reviewed after the discovery of ‘syngamy’ and ‘double fertilization’.
  - (2) In 1884 Strasburger discovered syngamy and Lemonnier (1887) suggested that the fusion of polar nuclei with second male gamete is equivalent to the act of fertilization. Hence, the endosperm is a tissue of sporophytic origin and is in fact second embryo modified for the nutrition of the first.
  - But this view was later on contradicted by embryologists because the endosperm did not always contain diploid chromosomes. The ploidy of the endosperm is very much variable and depends upon the number of polar nuclei, the number of male gamete always being one.
  - (3) Strasburger (1900) interpreted that ‘triple fusion’ acted as a growth stimulus. He considered the endosperm as a ‘belated gametophyte’ which developed only after double fertilization.
  - (4) Nawaschin (1898) announced the discovery of double fertilization and regarded triple fusion as normal fertilization, and therefore, the endosperm was said to be homologous to the normal embryo.
  - Based on Nawaschin’s conclusions, Miss Sargant (1900) suggested that the second embryo is not comparable to the normal embryo because the chalazal polar nucleus after fusion causes chromosomal imbalance.
  - Whatever is the nature of the endosperm, while all other ovular tissue have been supported to give rise to embryos, there is
no convincing report of the endosperm doing so, even as an abnormality.

**RUMINATE ENDOSPERM**
- A special type of endosperm has been reported in annonaceae, Myristicaceae, and some members of palmaceae and Rubiaceae. This is called ‘ruminate endosperm’
- Mature endosperm with any degree of irregularity and unevenness in its surface contour is called ruminate.
- It is said to arise as the result of invaginations of the outer tissue, namely seed coat, which penetrate deeper and eventually appear as wavy bands in the mature seeds.
- In psychotria (Fagerlind,1937) rumination is said to occur due to the activity of the endosperm itself which grows out and fills the ridges arising in the integuments in post fertilization stages.

**PROBABLE QUESTIONS**

Q. 1 What is endosperm? Write down the modes of its development in angiosperms.

Q. 2 Define triple fusion. what is the product of this process and what does the product develop into? Explain.

Q. 3 Describe different types of endosperm with suitable diagrams. Explain the morphological nature of the endosperm.

Q. 4 Write short note on –
   (i) Nuclear endosperm
   (ii) Cellular endosperm
   (iii) Helobial endosperm
   (iv) Ruminate endosperm
   (v) Endosperm haustoria
2. POLYEMBRYONY

- In angiosperms, normally only one embryo sac is present per ovule. so as a rule only one embryo is found per ovule after fertilization.
- But, reports of more than one embryo in an ovule is also not uncommon.
- After fertilization, the ovule is converted into seed.
- Occurrence of more than one embryo per seeds is termed as polyembryony.
- Leeuwenhoek (1719) was the pioneer who discovered polyembryony in orange seeds.
- Ernst (1918) and schnarf (1929) received the early literatures and classified polyembryony into two types (i) true and (ii) false.
- The adventive embryos which resulted in occurrence of polyembryony was termed as ‘true’ polyembryony.
- The aposporic embryo sacs in an ovule also results in occurrence of more than one embryo in a seed This type is designated as ‘False ‘ polyembryony.
- Gustafsson (1946) proposed that ‘false’ polyembryony should be restricted to only those cases in which two or more nucell fuse together at an early stage and the embryo sac of each nucellus gives rise to an embryo inside the same seed. All other cases should be included under ‘true’ polyembryony.
- On the basis of the origin of embryos in addition to the normal embryo, polyembryony is categorised into four types –

BOOK RECOMMENDED
(I) The embryology of angiosperms by Bhojwani and Bhatmagar
(II) An introduction to the embryology of angiosperm by P.maheshwari.
(III) college botany – vol. I by Ganguly and Kar
(i) Cleavage of proembryo
(ii) Embryos originating from cells of the embryo sac other than egg
(iii) Embryos arising from cells outside the embryo sac
(iv) Embryos originating from additional embryo sacs in the same ovule.

(1) **CLEAVEAGE POLYEMBRYONY**
- This is the simplest method of polyembryony is which increase in the number of embryos in two or more units takes place due to cleavage or splitting of the zygote or proembryo inside the embryo sac.
- This is commonly found in angiosperms also.
- Among angiosperms, it is frequently found in orchids.
Jeffrey (1895) gave the detailed account of cleavage polyembryony in Erythronium americanum. In this species, synergids degenerate and disappear after fertilization.

The zygote divides by a number of irregular divisions to form a disorganised mass of cells known as embryogenic mass. Later, protuberances appear at the chalazal end of this mass, which eventually grow into individual embryos.

Commonly, two to four embryos are distinguished.

Swamy (1943) reported three different modes of development of more than one embryo in Eulophia epidendraea:

1. The zygote divides to produce embryogenic mass. The cells at the chalazal end of the mass grow into a number of embryos.
2. The proembryo gives rise to a number of small bud like outgrowths which grow into embryos.
(3) The filamentous embryo becomes branched and each branch gives rise to an embryo, e.g. Vanda, an orchid, (Rao, 1965).

- Occasionally, the suspensor cell of the proembryo may also give rise to an embryo, e.g. exocarpus, a member of santalaceac (Ram, 1959).

(2) origin of embryos from the cells of the embryo sac other than egg cell.

- Inside the female gametophyte, syngamy between one male gamete and the egg cell results in normal embryo.
- But other cells of the gametophyte, viz. Synergids, antipodals and polar nuclei have also been reported to give rise to embryo.
- Among these cells, most common source of embryo are synergids, which may directly form the embryo or may be fertilized by the male gamete of the additional pollen tube.
• Presence of more than one male gamete or sperm is seen in many genera, e.g. cuscuta epithymum, Allium rotundum etc.
• The extra sperm may fertilize the same egg cell causing triploid embryo or it may fuse with one of the synergid forming another diploid embryo.
• Production of embryos from antipodal cells is sporadic. However, in ulmu americacas, allium odorum and a few other genera antipodal embryos have been reported.
• Embryos originating from polar nuclei and laterally located nuclei in the embryo sac are not confirmed. (Maheshwari and Johri, 1941).

(3) Embryo originating from cells outside embryo sac

* Strasburger (1878) for the first time observed that embryos can arise from the cells of the nucellus in addition to the normal embryo in orange seeds (Cibrus microcarpa, C. trifoliata
* Batchelor (1943) confirmed this observation.
* Juliano (1937) reported nuclear embryony in mango (Mangifera indica).
* Besides these popular examples, nucellar polyembryony is known to occur in genera like opuntia, eugenia spiranthes, trillium etc.

* the embryos, although originate outside the embryo sac, subsequently come to lie inside it and are nourished by the endosperm.

- In Nigritella nigra (Afzelius, 1928) the cells of the nucellar epidermis possess a remarkable capacity for growth and differentiation. One or two of them elongate considerably and form embryos by proliferation, budding or cleavage.
- In most of the cases, nucellar embryos arise from the micropylar half of the ovule.
- In Mangifera the nucellar cells destined to form adventive embryos can be distinguished from other cells of the nucellus by their dense cytoplasm and starchy contents.
In Euonymus, epidermal and sub epidermal cells of the inner integument proliferate in the formation of embryos.

(4) Embryos arising from other embryo sacs in the ovule.

* Sometimes more than one embryo sacs are seen in an ovule.

* Multiple embryo sacs may arise-----

(i) either from the derivatives of the same megaspore mother cell.

(ii) from two or more MM Cs , or

(iii) from nucellar cells without meiosis (Aposporic embryo sacs).

- Bacchi (1943) found more than one embryo sac in the ovule of citrus resulting in two zygotic embryos.

- Nielsen (1946) reported the same in Poa pratensis.

Conclusion

- Whatever be the mode of origin of embryo in an ovule, only one reaches maturity, all others degenerate either at an early stage or as a result of mutual competition.

- Polyembryony, although fairly widespread in angiosperms, is much less common in them than it is in the gymnosperms, the reason for this is that in the latter there are several archegonia in a gametophyte each having an egg cell whereas in the former only one egg cell is present in an ovule.

Probable questions-

(1) What do you understand by polyembryony ? what are the modes of its occurrence in angiosperms?

(2) What are methods by which polyembryony oceurs in an angiosperm? What is its importance.

(3) Write short note on cleavage polyembryony.

Books recommended

(1) The embryology of angiosperms by Bhojwani and Bhatnagar.
3. APOMIXIS

INTRODUCTION

- The life cycle of a higher land plant, viz. pteridophyta, Gymnosperms and Angiosperms shows alternation of generations and is completed by two morpologically dissimilar generations:
  (i) The sporophyte and
  (ii) the gametophyte.
- The vegetative plant body is sporophytic in nature
- The gametophyte is produced at the time of reproduction and is of very short duration in the life-cycle.
- The sexual reproduction involves two processes (a meiosis and (b) syngamy.
- After meiosis, male and female gametophytes are produced on the sporophyte.
- Male gametophyte is represented by pollen grain in which two male gametes are present.
- Female gametophyte is represented by embryo sac present in the ovule.
- Inside the embryo sac eight haploid nuclei are present in which egg cell is the normal female gamete.
- Syngamy of haploid male and female gametes results into a diploid cell Known as zygote.
- This process is called Amphimixis.
- Besides amphimixis, plants may also produce embryos with or without involvement of gamete.
- This process is called apomixis.
Winkler (1934) defined apomixes as a substitute of sexual reproduction in which syngamy does not occur. This is also called agamospermy.

The plants in which sexual reproduction is completely replaced by apomixes are called apomictic.

It has been reported that both amphimixis and apomixes may occur in the same plant species. However, former may be found in certain part of the world and latter in another part.

This process may be classified into four types. –
1. Vegetative reproduction
2. Non-recurrent apomixes
3. Recurrent apomixes
4. Adventive embryony

VEGETATIVE REPRODUCTION

Propagation of a plant from any part other than seed is called vegetative reproduction.

The part which produces new plant is called propagule. These may be bulbies, tubers, adventitious parts etc.

Gustafsson (1946) has distinguished three types of vegetative reproduction.

(i) Despite the presence of functional sex organs, no syngamy occurs failing in the production of seed. Propagules are formed outside the floral region. E.g. Agave americana.

(ii) plants have flowers, but they are sterile. Propagation takes place by means of bulbils, bulbs, rhizomes etc. e.g. Fritillaria imperialis, Lilium bulbiferum etc.

(iii) the propagules are formed on the floral branches in addition or in place of them. This phenomenon is commonly known as vegetative.

Vivipary e.g. many grasses.

NON-RECURRENT APOMIXIS

This type is also called gametophytic apomixes.
The megaspore mother cell (MMC) undergoes normal meiosis resulting into a haploid embryo sac.

The embryo may be formed from the haploid egg cell, the phenomenon be called as haploid parthenogenesis. e.g. solanum nigrum.

The embryo may be generated from any cell of the embryo sac other than egg cell such as antipodals synergids, polar nuclei etc, the phenomenon being termed as haploid apogamy. eg. Lilium martagon.

Since the embryos produced by this method are haploid, they, remain sterile and the process is not carried from one generation to another.

**RECURRENT APOMIXIS**

- Other name for this type is sporophytic apomixes.
- This type is characterised by absence of meiotic division or its failure during various phases of prophase I or other phases of meiosis I and II in the MMC.
- This method is termed as generative apospory or diplospory.
- Failure of meiosis results in the formation of a special type of nucleus known as restitution nucleus.
- The restitution nucleus is formed when two sets of chromosomes after anaphase of division is enveloped in the same nuclear membrane.
Initially, this nucleus is dumb bell shaped, but later on it becomes spherical e.g. Ixeris, dentata, Eutatorium glandulosum.

Formation of diplosporic embryo sac in Ixeris dentate

Origin of embryo sac from any cell of the nucellus other than megaspore mother all is termed as somatic apospory.

Embryos in the aposporic embryo sac arises from the egg cell, the process being known as diploid parthenogenesis.

Embryos may arise form some other cells of the diploid embryo sac, the process being known as diploid apogamy.
ADVENTIVE EMBRYONY

- When the embryos arise from the diploid cells lying outside the embryo sac and belonging either to the nucellus or the integument, the process is called adventive embryony.
- There is no alternation of generation.
- The gametophytic generation is completely eliminated.
- A common feature of this process is that the cells concerned in such development become richly cytoplasmic and divide actively to form small group of cells which eventually push their way into the embryo sac and grow further to form true embryos.
- Frequently the zygotic embryo also develops simultaneously, e.g. Citrus trifoliata (strasburger, 1878), Mangifera indica (Juliano, 1937).
- In Nigritella nigra (Afzelius, 1932), an orchid, nucellar epidermis is reported to give rise to an embryo.
SIGNIFICANCE OF APOMIXIS

- There are two types of apomictic plants: (i) obligate apomicts and (ii) facultative apomicts,
- Obligate apomicts are those plants which lack normal sexual reproduction despite the presence of functional reproductive parts. These plants do not show evolutionary flexibility.
- Facultative apomictic plants have both true zygotic embryos and the apomictic embryos which coexist. Hence, chances of variation and evolution is more categorical.
- This phenomenon gives the opportunity of possibility for indefinite multiplication of favourable biotypes without any variation due to segregation or recombination.

Probable question-

1. What do you mean by apomixes? Describe different methods for generation of plants without reproduction.
2. Distinguish between amphimixis and apomixes. How are embryos produced apomictically?
3. Give an account of various types of apomixes with suitable examples. What is its significance?
4. Write short notes on: (i) Non-recurrent apomixes (ii) Recurrent apomixes (iii) adventive embryony (iv) agamospermy.

Books recommended –(1) the embryology of angiosperms by Bhojwani and bhatnagar (2) an introduction to the embryology of angiosperms by p. maheshwari (3) college Botany vol. I by ganguly & kar.
4. FEMALE GAMETOPHYTE OR EMBRYO SAC

INTRODUCTION:

- In angiosperms, each ovule inside the ovary consists one female gametophyte, also known as mega gametophyte or embryo sac.
- In a few cases, more than one embryo sac has also been reported (sedum sp.) but only one remains functional.

DEVELOPMENT

- Just beneath the nuclear epidermis single cell differentiates as ARCHESPORIUM.
- Occasionally, more than one archesporial cells are also seen. e.g. Hydrilla.
- Archesporium may directly turn into megaspore mother cell (MmC) or may divide periclinally in two cells outer cell by further division adds to the wall of the nucellus and the inner cell acts as MMC.
- The MMC divides meiotically and gives rise to four haploid megaspores.
- Out of four megaspores only the chalazal megaspore remains functional.
- Three micropylar megaspores degenerate.
- The functional megaspore enlarges considerably and forms a sac like cavity embedded in the nucellus.
- Single haploid nucleus in this cavity divides mitotically thrice and gives rise to eight nuclei.
- These nuclei migrate towards the micropylar and chalazal ends having four nuclei each.
- The cytoplasm is displaced towards the peripheral region.
- The central part of the cavity is occupied by a large vacuole. One nucleus from each end shifts towards the centre. These centrally placed nuclei are called polar nuclei.
- Each nucleus becomes surrounded by a small amount of cytoplasm enveloped by wall.
• There cells at the micropylar end forms the egg apparatus having two synergids and an egg cell.
• The other three at the chalazal end are called antipodal cells.
• This is the normal type of embryo sac and is designated as polygonum type.

MATURE EMBRYO SAC
• the megagametophyte is an eight–nucleate oval structure consisting of a large central vacuole and cytoplasm pushed towards the periphery adhered to the wall.
• Out of eight nuclei, three are present at the micropylar end, become enveloped by wall individually and constitute the egg apparatus.
• The egg apparatus has two cells called synergids and one egg cell.
• Three nuclei present at the chalazal end are known as antipodal cells.
• Two nuclei at the centre of the cavity either remain free or become enveloped by a common wall and are called secondary polar nuclei.
• About 81% of the angiospermic plants have anatropous ovule having micropyle parallel and close to the funicules.
Hence the lower end of the female gametophyte is micropylar end and upper end is chalazal.

**TYPE OF FEMALE GAMETOPHYTE**

- The normal or polygonum type of embryo sac is derived from one megaspore lying at the chalazal end.
- This type is called Monosporic megagametophyte.
- But participation of two as well as four megaspores has also been reported.
- These are designated as bisporic and tetrasporic embryo sacs respectively.
- Each type has more than one subtypes named after the genus in which it was first discovered.

**MONOSPORIC EMBRYO SAC**

- There are two subtypes of monosporic embryo sac.
- (1) polygonum type – this is the normal embryo sac developed from the chalazal megaspore.
  - This is an eight nucleate embryo sac.
- (2) Oenothera type- this type is characterized by absence of third mitotic division in the haploid megaspore nucleus.
  - The micropylar megaspore is functional and the mature embryo sac has only 4 nuclei.
  - These nuclei at the micropylar end form the normal egg apparatus, one nucleus at the centre forms the polar nucleus.
  - Antipodal cells are absent. Eg. Members of onagraceac.
BISPORIC EMBRYO SAC

- This type of embryo sac is characterized by the involvement of two megaspores in the formation of gametophyte.
- The megaspore mother cell (MMC) divides by meiosis I into two haploid cells. Each being known as a dyad cell.
- Meiosis II is absent in one of these dyad cell and ultimately it degenerates. So, two megaspores do not exist.
- The other dyad cell divides by meiosis II and two haploid megaspore nuclei are formed which lie in the same cavity. Both these megaspore nuclei participate in the formation of megaspore, hence the name bisporic.
- This type has also two subtypes:
  - **Allium type** - the micropylar dyad cell degenerates.
  - The chalazal dyad cell is functional.
  - it enlarges and forms 8-nucleate embryo sac, nuclei being organized like polygonum type.
(2) Endymion type- this is also eight nucleate, the only difference from the Allium type is that it is developed from the micropylar dyad cell.

**TETRASPORIC EMBRYO SAC**

- In tetrasporic embryo sac neither of the meiotic division is accompanied by wall formation. 
- At the end of the meiosis all the four megaspore nuclei lie in the common cytoplasm forming a coenomegaspore. 
- All the nuclei participate in the formation of the embryo sac, hence the name tetrasporic. 
- As the four nuclei are the products of the same meiosis, they are genetically different. 
- Before megagametogenesis, the nuclei of the coenomegaspore migrate to a particular position, then divide mitotically, 
- The mitotic division may occur once or twice resulting in eight or sixteen nuclei respectively. 
- Arrangement of nuclei in coenomegaspore is of three types: 
  - (a)1+1+1+1, in which one nucleus is present at the micropylar end, one at the chalazal end and one each on two lateral sides of the embryo sac.
(b) 2+2, in which two each are present at the micropylar and chalazal ends.

(c) 1+3, in which one nucleus is placed at the micropylar end and three at the chalazal end. Three nuclei at the chalazal end may remain free (ci) or may be enveloped in a common wall forming a triploid cell (ciii).

Depending upon the arrangement of nuclei, number of mitotic division and whether nuclear fusion in the coenomegaspore occurs or not, the tetrasporic embryo sac are of various types:

(A) Nuclear fusion does not occur

(i) Adoxa type – the arrangement of nuclei is 2+2 type and mature embryo sac looks like polygonum type with 8 nuclei.

(ii) Plumbago type – 1+1+1+1 arrangement of four megaspore nuclei results into two nuclei each at micropylar end, chalazal end and both lateral sides. One nucleus from each side shifts to the centre making 4-nucleate secondary polar nuclei.

(B) Nuclear fusion occurs two mitotic divisions occur

(iii) Panacea type – 1+1+1+1 arrangement of four nuclei results into four nuclei each at micropylar end, chalazal end and both lateral sides, Mature embryo sac has three nuclei on each side and four nuclei at the centre.

(iv) Peperomia type – the arrangement is 1+1+1+1, two cells constitute the egg apparatus, six antipodals and 8 nuclei are present at the centre, the embryo sac is 16-nucleate,
• **(v) Drusa type**- the embryo sac is 16-nucleate with 3-celled egg apparatus, 11 antipodals and 2 polar nuclei. Arrangement is 1+3 type.

II. **Nuclear fusion occurs.**

• **Fritillaria type**- the arrangement is 1+3 type. Three nuclei at the chalazal end fuse together and form a triploid nucleus, Micropylar haploid nucleus and triploid chalazal nucleus divide twice and each end have four nuclei each, Mature embryo sac has three haploid cells forming egg apparatus, three triploid antipodal cells and one haploid and one triploid nuclei as secondary polar nuclei

• **(vii) plumbagella type**- the arrangement is 1+3 type.
After nuclear fusion at the chalazal end, only one mitotic division occurs at each end and two haploid and two triploid cells are produced. Mature embryo sac consists of only one egg cell without synergds, one triploid antipodal cell, one haploid and one triploid secondary polar nuclei.

**Special type**-

- A peculiar type of tetrasporic embryo sac has been discovered by Martinoli(1939) in chrysanthemum cinerariae folium. In this type, the arrangement is 1+2+1 type, one nucleus each at the micropylar and chalazal end and two nuclei at the centre.
- The micropylar and chalazal nucleus divides further, but the central nuclei do not divide.
- Central nuclei may remain free or may fuse together. Hence, the organization of the nuclei in the embryo sac varies.

In addition to these distinct and well established types of embryo sacs, many isolated types have also been reported which are not common.
**Probable questions**

Q.1. what do you mean by mega gametophyte? Write down the modes of development of mega gametophytes.

Q.2. Give an account of the types of embryo sac with suitable diagrams.

Q.3. write down the various types of development of female gametophyte. What is the fate of secondary polar nuclei in each type.

Q.4. write short notes on-

(i) Monosporic embryo sac

(ii) Bisporic embryo sac

(iii) embryo sac of chrysanthemum

(iv) structure of embryo sac

**Books recommended**-

1. An introduction to the embryology of angiosperms - P. Maheshwari

2. The Embrology of angiosperms - Bhojwani and Bhatnagar

3. College Botany vol. I – Ganguly and kar
Dr. Dilip Kumar Jha
Assistant Professor of Botany
C.M.Sc. College, Darbhanga
1. Principles and techniques of tissue culture.

**Principles and techniques**

Plant tissue culture is defined as the maintenance and growth of plant cells, tissues and organs on a suitable culture medium in vitro, e.g., in a test tube or any other suitable vessel, in aseptic condition. The technique of plant tissue culture have become important tool for crop improvement, commercial production of natural compounds and in the development of forestry. It occupies a key role in the green revolution.

Haberlandt, The father of plant tissue culture, in 1898 tried to culture the growing cells. Hanning(1904) cultured the matured embryo of genus *Raphanus* and obtained the plantlets. Knudson(1922) cultured the seed of orchids. F.C. Steward(1932) developed a complete carrot plant from root cell.

**Cellular Totipotency**

This is the ability of mature cells to grow into a new individuals. Every living plant cell, irrespective of its age and location, is totipotency. Steward(1932) was the first to recognize the capacity in carrot root cells. Guha and Maheshwari(1964) also noticed it while culturing the anther of *Datura innoxia* on basal medium. They obtained Laploid Plantlets.

**Explants**

An explant is the part of a plant that is excised from its original location and used for initiating a culture. Thus, plant tissue cultures are classified on the basis of in vitro growth or on the basis of explants used e.g. callus and suspension culture, Embryo culture, anther culture, Meristem culture, protoplast culture, Nucellus and ovary culture, endosperm culture etc.
Sterilization

It is essential that the explants, glassware, culture containers or vessels, media and the instruments used for plant tissue culture must be free from microbes.

The explants are treated with specific anti-microbial chemicals, and the processing called surface sterilization. Suitable sized plant material (explants) is sterilized as follows:

- The explants to be sterilized is brought-in well sterilized laboratory are used sterilization.
- Working area and hands are cleaned with alcohol, is added mask and cap are used and light the spring lamp.
- 3 or 4 petridishes are kept in line, disinfectant is added (e.g. mercuric chloride or 20% sodium chloride) in first plate and autoclaved distilled water in subsequent plates.
- Plant pieces are placed in first plate and the material is immersed with the help of sterilized forceps for 5-10 minutes depending upon the disinfectant used.
- Material is transformed from first to second petripate, rinsed gently and passed to third and fourth plates, one by one with thorough rinsing.
- Finally the distilled water is drained and suitable sized explants are prepared.

A quick dip in 70% ethanol (15-30 seconds), is always advantageous, before surface sterilization with disinfectant.

The vessels, media and instruments are also suitably treated with steam, dry heat, alcohol and subjected to filtration to make them free from microbes. Generally, autoclave is used to sterilize medium, glassware and tools for the purpose of plant tissue culture. Sterilization of material is carried out by increasing moist heat (121°C) due increase pressure inside the vessel (15-22 psi, i.e. pounds per square inch) for 15 minutes for routine sterilization. Moist heat kills the microorganisms and makes the material free
from microbes. Surface sterilization of explants and their transfer to culture media must be done under aseptic conditions.

**Culture Technique**

The technique of plant tissue culture enables us to study the cell tissue or organs by isolating them from the plant body and growing aseptically, in suitable containers, on an artificial nutrient medium, under controlled environmental conditions. Thus, (i) nutrient medium, (ii) aseptic conditions and (iii) aeration of the tissue are important aspects of the technique of in vitro culture.

The important aspects of techniques of in vitro plant tissue culture are:-

(i) Nutrient medium
(ii) Aseptic conditions
(iii) Aeration of the tissue

**Culture medium or Nutrient medium**

When plants are artificially grown in laboratory, they depended upon the nutrients supplied by the medium. This is called culture medium.

Different plant species has its special requirement of nutrient for optimal growth. Thus, different types of culture media have been developed by scientists. Some important c media are:-

1. White’s Medium (WM)
2. Murashige and Skoog Medium (MS)
3. Nitsch Medium (NM)
4. Nagata and Tabake Media (NT)

**Table I- Different type of Culture Media**

<table>
<thead>
<tr>
<th>Inorganic Compounds</th>
<th>WM</th>
<th>MS</th>
<th>NM</th>
<th>NT</th>
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</thead>
<tbody>
<tr>
<td>Potassium chloride KCl</td>
<td>65</td>
<td>--</td>
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<td>--</td>
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<tr>
<td>Potassium nitrate KNO₃</td>
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<td>1900</td>
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<tr>
<td>Chemical Name</td>
<td>Formula</td>
<td>C</td>
<td>H</td>
<td>N</td>
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<td>-------------------------------</td>
<td>-----------------------------</td>
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</tr>
<tr>
<td>Ammonium nitrate</td>
<td>NH₄NO₃</td>
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<tr>
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<td>Cobalt sulphate</td>
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<td>--</td>
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Most of the medium contain inorganic salts of major and minor elements, vitamins and sucrose. A medium with these ingredients will be referred to as basal medium. Sometimes, growth regulators, such as auxin gibberellins and cytokinins, may also be added to the basal medium. Growth regulators are required for cell division and organ regeneration from the cultures. The cultures are usually kept in a culture room at about 24°C with some illumination. These all constituents are dissolved in distilled water. If necessary, the medium is solidified with about 0.8% agar. The pH of the medium is adjusted around 5.8 (slightly acidic). Now equal quantities of the medium are dispersed in culture vials, which are usually glass tubes or flasks. The culture vials, containing medium are plugged with non-absorbent cotton wrapped in cheese cloth. Such a closure allows the exchange of gases but does not permit the entry of micro-organisms into culture vials.

**Aseptic Conditions**
The sugar content of the nutrient media may support a luxuriant growth of many microorganisms, like bacteria and fungi. It is,
therefore, extremely important to maintain a complete aseptic environment inside the culture vials. Micro-organisms can contaminate the medium in at least three ways:

(a) The micro-organisms present in the medium right from the beginning may be destroyed by sterilizing the properly plugged culture vials. It can be done by maintaining the temperature above 120°C for about 15 minutes.

(b) The micro-organisms may also be carried along with tissue that is being cultured. To prevent this, the plant material from which the tissue is to be excised is surface sterilized. The material may be surface sterilized with saturated chlorine water and then thoroughly washed with sterilized distilled water and to remove all traces of chlorine. If the material is fairly hard, as some fruits and seeds, it may be surface sterilized by rinsing in alcohol.

(c) Finally, precautions must also be taken to prevent the entry of microorganisms while the plug of a culture vial is removed to transfer the tissue to the nutrient medium (inoculation). For this, all operations from surface sterilization of the tissue up to inoculation are done in an aseptic environment.

Aeration

Proper aeration of the cultured tissue is also an important aspect of culture technique. If the tissue is grown on the surface of a semi-solid medium it acquires enough aeration without special device for liquid medium, special device “filter paper bridge” is used. Here, two legs remain dipping in the medium and the horizontal part carrying the tissue is raised above the level of the medium.
Applications of Tissue Culture

a. The technique provides a way for rapid multiplication of desirable and rare plants.

b. Reveal, we may obtain healthy stocks from virus infected plant through shoot-tip culture.

c. The development of haploids through the techniques of anther culture is having us potential significance in basic and applied genetics and plant breeding.

d. Embryo culture has been useful in overcoming seed dormancy. It is also utilized for producing viable plants from crosses which normally fail due to the death of immature embryo. e.g., jute, rice.

e. The embryo tissue culture is also applied for the propagation of rare plants.

f. Another important use of embryo culture is in obtaining some rare hybrids. It is possible to raise complete hybrid plants through embryo culture. This method has been profitably used for many interspecific crosses.

Culture Technique

Explant culture. There are a variety of forms of seed plants, such as trees, herbs, grass, which exhibit the basic morphological units, i.e., root, stem and leaves. Parenchyma is the most versatile of all types of tissues. They are capable of division and growth. Development of a tissue is characterised by (i) cell division, (ii) cell elongation and (iii) cell differentiation. For this reason, the explant from healthy and young part of the plant is used. Presence of parenchyma is first consideration in a particular species. parenchyma from stems, rhizomes, tubers, root is easily accessible and will generally respond quickly to culture conditions in vitro.
**Callus formation and its culture**- A Callus is an amorphous mass of loosely arranged thin walled parenchyma cells developing from proliferating cells of the parent tissue. In nature, callus develops by infection of microorganisms from wounds due to stimulation by endogenous growth hormones, the auxins and cytokinins. However, it has been artificially developed by adopting tissue culture techniques.

In callus culture, cell division in the explant forms a callus, an unorganised mass of cells. It is maintained on a medium gelled usually with agar. The medium ordinarily contains the auxin 2, 4-D (2, 4-dichlorophenoxyacetic acid), and often a cytokinin HAP (benzylaminopurine). When an explant is placed on such a medium, many of the cells become meristemetic and begin to divide. In about 2 to weeks, a callus mass is obtained.

**Cell (suspension) culture**- A suspension culture consists of single cells and small groups of cells suspended in liquid medium. Cell suspension is prepared by transferring a fragment of callus about(500mg.) to the liquid medium (500 ml.) and agitating them aseptically to make the cells free in medium. The medium ordinarily contains the auxin 2, 4-D. Suspension cultures must be constantly agitated at 100-250 rpm (revolution per minute).

Agitation serves three important purposes. They are as follows

(i) aeration of culture (ii) constant mixing of the medium and (iii) breakage of cell aggregates into smaller cell groups.

Suspension cultures grow much faster than callus cultures.

It is difficult to have suspension of single cell. However, the suspension includes single cell, cell aggregates (varied number of cells), residual inoculum and dead cells. King (1980) has described that a, good suspension consists of a high proportion of single cells than small cluster of cells.

Cell suspension cultures have many advantages over the callus cultures. They are as follows.
(i) The suspension can be pipetted.

(ii) They are less heterogeneous and cell differentiation is less pronounced.

(iii) They can be cultured in volumes upto 1500 litres.

(iv) They can be subjected to more stringent environmental controls.

(v) They can be manipulated for production of natural products by feeding precursors.

**Subculturing** - After some time, the undermentioned three things happen in all types of plant tissue cultures.

(i) Cells/tissues dry matter known as biomass increases, (ii) the level of nutrients in the medium decreases, and (iii) the medium volume declines due to evaporation.

Hence, if tissue cultures were kept in the same culture vessel, they will die in due course of time. Due to this reason, cells/tissues are regularly transferred into new culture vessels containing fresh media. This process is called subculturing.

Precaution is taken that during subculture, only a part of the culture from a vessel is the new culture vessel.

**Uses of callus and suspension cultures** - The callus and suspension cultures can be used to achieve cell biomass production which may be used for biochemical isolation.

Also used for regeneration of plantlets, *i.e.*, newly regenerated plants through tissue culture.

Production of transgenic plants and isolation of protoplasts.

**Regeneration of Plantlets.**

Regeneration describes the development of an organised structure, such as root, shoot or somatic embryo from cultured
cells. However, plantlets can be obtained from cultured cells by two different ways:

(i) **Shoot regeneration** is followed by rooting of the shoots and

(ii) **Regeneration of somatic embryos** followed by their germination.

In cultured materials it has been possible to study such process as differentiation of a parenchyma cell into tracheid (*i.e.* cytodifferentiation) **organ formation** (*i.e.*, organogenesis) and somatic embryo formation (*i.e.* Somatic embryogenesis).

**Organogenesis**- The process by which cells and tissues are forced to undergo changes which lead to the production of a structure, known as shoot or root primordium.

**Advantages of plantlet regeneration**- There are several advantages of planlet regeneration through plant biotechnological methods using organogenesis or embryogenesis, in comparison to conventional methods of propagation. The advantages include the efficiency of process, i.e. formation of plantlet in fewer steps, with reduction in labour, time and cost, and the potential to the production of much higher number of plantlets, and their morphological and cytological uniformity. Till now about 150 species from angiosperms and gymnosperms have been reported to produce somatic embryos in culture.

**Shoot and root regeneration**- Shoot regeneration is promoted by a cytokinin, such as BAP (benzylaminopurine), while root regeneration is promoted by an auxin, such as NAA (naphthalene acetic acid). Thus, shoot and root regenerations are generally controlled by auxin-cytokinin balance. Usually, an excess of auxin promotes root generation, and that of cytokinin promotes shoot generation.

Callus cultures are first kept on a BAP (cytokinin) containing medium. After sometime shoots, regenerate from callus cells, when the shoots become 2-3 cm long, they are excised and
transferred to an auxin-containing medium. After sometime, roots regenerate from the lower ends of these shoots to give rise to complete plantlets.

**Somatic embryo regeneration**- process of development of embryo is called embryogenesis. Normally embryo develops from zygote formed due to fertilization. Sometimes diploid somatic cells of the plants can develop into embryo is called “somatic embryo”. It is a nonsexual developmental process. Artificial seed is produced by mounting the somatic embryo in alginate.

Steward and Reneirt(1958) reported for the first time, somatic embryo formation in carrot cell suspension cultures. These somatic embryos were similar to zygotic embryos in development and structure.

A somatic embryo develops from a somatic cell. The pattern of development of a somatic embryo is similar to that of a zygotic embryo.

Somatic embryo regeneration is induced usually by a relatively high concentration of an auxin like 2, 4-D (2, 4-dichorophenoxyacetic acid). The young embryos develop into mature embryos either in the same medium or on another medium. Mature somatic embryos germinate to yield complete plantlets.

During somatic embryo generation in cell suspension cultures, embryos of different sizes are produced. For any experimental or micropropagation method, embryos of uniform size are required. This can be achieved by sieving or fractionation of suspension with appropriate sieve size.

Somatic embryo regeneration is a versatile technique for micropropagation of plant species. A large number of herbaceous dicots and monocots have been regenerated through somatic embryogenesis.
Establishment of plantlets in the field- Plantlets are produced through rooting of isolated shoots or germination of somatic embryos. Now, the plantlets can be removed from culture vessels and established in the field. This stage is concerned with transfer of plantlets in pots, their hardening and establishment in soil. Hardening of plants imparts some tolerance to moisture stress and plants become autotrophic from heterotrophic condition. During hardening, plantlets are kept under a reduced light and high humidity for a suitable period of time. Hardening procedures make the plantlets capable of tolerating the relatively harsher environments outside the culture vessels.

Hardened plants are then transferred to glass or poly-houses with normal environmental conditions. Generally the poly-houses are erected by mounting polythene or polycarbonate sheets on metal frame support. Now, plants are irrigated frequently and their growth and variation monitored regularly. Plants are generally transferred to fields, e.g., plantation crops, after 4-6 weeks of acclimatization.

In India, there are now many commercial companies which produce millions of plantlets through micropropagation.

Large glass houses and green houses are essential components of micropropagation industry. Hardening and acclimatization of delicate *in vitro* raised plantlets is carried out in these glass houses. Now a days chambers made of polycarbonate and polypropylene sheets arc used for creating large working place. These houses are fitted with mist and fog generating units with cyclic auto-regulation. Light is provided through proper light sources.
Line Diagram to show development of whole plant by Callus and Suspension Application of plant tissue culture. Tissue culture has been successfully employed for the multiplication of orchids and many other ornamental plants.
In the developed countries tissue culture is a routine method of multiplication while in developing countries, such as India, the techniques are largely used for producing plants for export markets.

However, practical applications of plant tissue culture are mainly based on the ability of plant cells to give rise to complete plantlets. The use of plant cells to generate useful products is called plant biotechnology. In most of its activities, the useful product is plantlet that, in many cases may have been genetically altered. Usually, these plantlets are used for the following important purposes:

(i) Clonal propagation. Clonal propagation by vegetative methods is a practice followed since man started cultivation of plants. The main objective of clonal propagation has been to reproduce plants of selected desirable qualities uniformly and in bulk. The traditional propagation methods, require long duration, whereas tissue culture helps in rapid plant multiplication.

A clone is a group of individuals or cells derived from a single parent individual or cell through a asexual reproduction. All the cells in callus or suspension culture are derived from a single explant by mitotic division.

Hence, all plantlets regenerated from a callus or suspension culture generally have the same genotype and constitute a clone.

These plantlets can be used for rapid clonal propagation of superior lines.

Selected examples of clonal multiplication of trees and horticulture plants are as follows oil, palm, citrus peach, prunus, poplar, etc. Improved cultivars developed by biotechnological methods are then clonally multiplied to replace inferior cultivated varieties, e.g., in Mentha and other aromatic plants.
(ii) Somaclonal variation. Genetic variation present among plant cells of a culture is called Somaclonal variation.

4. Embryo Culture

Interspecific crosses may fail due to several reasons, but when the development of embryo is arrested owing to the degeneration of the endosperm, or when the embryo aborts at an early stage of development, embryo culture is the only technique to recover hybrid plants. It is used extensively in the extraction of haploid barley.

Embryo culture is also a routine technique employed in propagation and inbreeding of those species that show dormancy. Das and Burman (1992) developed the method of regeneration of tea shoots from embryo callus.

Excision of young embryos from developing seeds and their cultivation on a nutrient medium is called embryo culture. *In vitro* older embryos are more easily cultured than young embryos.

The objective of embryo culture is to allow the young embryos to complete development and ultimately, give rise to seedlings.

More recently, a number of hybrids been successfully raised through embryo culture. *Hordeum Vulgare x Secale Cereale, Hordeum Vulgare x Agropyron repens, Hordeum Vulgare x Triticum aestivum* interspecific hybrids in *Abèlmoschus* and *Secale*. 
In some interspecific crosses, the endosperm of developing hybrid seeds degenerates at an early stage. Here, young embryos also die on the degeneration of endosperm. Hence such interspecific crosses cannot be normally made. In such cases, young hybrid embryos are excised and cultured in vitro to obtain hybrid seedlings.

The orchid industry owes a great deal to the technique of embryo culture. The culture of orchid embryos was initiated at the Singapore Botanic Garden in 1928. Orchids lack stored food. Here embryo culture allows development of seedlings from most of the embryos. In such cases, embryo culture is also used for rapid clonal propagation.

Embryo culture has helped in overcoming self-sterility of seeds, especially of crop plants propagated vegetatively, when the seeds do not germinate in nature. In a wild relative of commercial banana, Musa balbisiana, the seeds do not germinate under natural condition. However, if the embryos are excised and grown on a simple culture medium seedlings are readily obtained.

Rangaswamy and Rangan (1963) have cultured embryos of a stem parasite, Cassytha in the absence of the host, on a modified White’s medium supplemented with IAA. The embryos Cuscuta reflexa, a total stem parasite, were also cultured. Somewhat similar results have been obtained with the embryos of Dendrophthoe falcata, a partial stem parasite. Like the embryos of stem parasites, those of some root parasites have also shown, considerable capacity for. In cultures of seeds of Orobanche aegyptiaca, a total root parasite, the ovoid organized embryos produced a massive callus capable of continuous growth followed by differentiation into shoot tips.
2. Haploid Culture (Anther and Ovule Culture)

In Nature Haploid plants originate from unfertilized egg cells. However, in laboratory they are produced from both male and female gametes.

Anther Culture

Anther is a made reproductive organ. It is diploid in chromosome number. Haploid microscopes or pollengrains are formed by meiosis in spore mother cell (2n). the process is called Microsporogenesis.

In many plants, haploids by produced when their anthers are cultured on a suitable medium. This process is called anther culture. Best method of haploid production is pollen culture. This is called androgenesis (Tulecke, 1958 in Ginkgo biloba). Guha and Maheshwary (1964) successfully obtained embryo like structure from (Embryoids) from anther culture in Datura innoxia. Investigations have shown that for some reason, anthers from flowers of the Solanaceae respond best to excision and culture by production of embryoids. Convincing demonstration of the direct transformation of microspores into embryoids has been provided by several workers in different species of the genera of Solanaceac, i.e., Datura, Nicotiana, Afropa, Lycium, Solanum, Capsicum and Hyoscyamus. It has been observed that embryoids go through the globular, heartshaped and torpedo stages typical of the ontogeny of normal diploid zygotic embryos before they finally elongate and form shoot and root meristems. Embryoids have also been obtained form cultured anthers of certain cereals like rice.

In some other plants, haploids do not arise directly from the microspores, but do through the intervention of a callus. The microspores first develop into multicellular bodies which later give rise to an exceedingly dense callus. Subculture of the callus in an embryogenesis-inducing medium containing specific concentration
of auxins and a cytokinin led to the initiation of haploid seedlings. There, thus seem to exist the following alternative pathways for haploidy in anther cultures:

1. Microspore $\rightarrow$ callus $\rightarrow$ embryogenesis $\rightarrow$ plantlet e.g., Solanaceae

2. Microspore $\rightarrow$ callus $\rightarrow$ embryogenesis $\rightarrow$ plantlet e.g., Oryza, Brassica, Hordeum,; Coffea, Populus.

3. Microspore $\rightarrow$ callus $\rightarrow$ plantlet, e.g., Datura meteloides.

In several species, the pollen grains can be isolated and cultured to obtain haploids. In many species of plants, haploids can also be produced by culturing unfertilised ovaries or ovules.

Applications- Haploids, such produced are completely sterile and no direct value. But they are important as they are used to produce homozygous lines in 2-3 years. This strategy can be of superb value in breeding programmes. Anther from F$_1$ plants, obtained by crossing two or more species are cultured to obtain haploid plants.
The chromosome number of haploid plants may be doubled using colchicines to obtain homozygous plants. They progeny from these plants are then used to isolate superior homozygous lines. As these plants have single set of gene, somatic change can be detected.

At present, more than 347 plant species and hybrids belonging to 38 genera and 34 families of dicots and monocots have been regenerated using anther culture technique. They include economically important crops and trees, such as rice, wheat, maize, coconut, rubber plantation etc.
Culture of Ovules and Parts of Ovules

White (1932) was the pioneer to culture the ovules. White and La Rue cultured ovules of and antirrhinum, on White basic medium containing indole acetic acid IAA.

Nirmala Maheshwari and Lal (1961) cultured the ovules of Papaver somniferum excised six day after pollination when they contained only a 2-celled proembryo and a free nuclear endosperm. These grew to maturity in twenty days and even germinated and produced seedlings in the culture tubes.

Several genera, such as Citrus, Eugenia and Mangfera show the occurrence of nuclear embryos. Since they have the same genetical composition as the maternal parent, they are of much importance for the clonal propagation of desirable varieties. Rangaswamy (1961) reported that if nuclear tissue of Citrus microcarpa is grown on a suitable nutrient medium containing casein hydrolysate, it proliferates profusely and on subculturing produces embryo-like regenerants formed “pseudobulbils”, which can develop into seedlings so that an indefinite number of new plants can he obtained from a single nucellus.

A. N. Rao (1963) successfully germinated seeds of an interspecific hybrid of Vanda on a simple agar nutrient medium. Some of the fertilized ovules directly produced seedlings; others gave rise to a callus from which new plants arose subsequently.

3. Protoplast culture and somatic Hybridisation

Protoplast culture involves the culture of isolated protoplast by breaking down the cell wall mechanically or chemically using enzymes. In other words, the cells are rendered naked and cultured.
When cell wall is mechanically or enzymatically removed, the isolated protoplast is known as ‘naked plant cell’ on which most of recent researches are based.

Plant cell wall acts as physical barrier and protects cytoplasm from microbial invasion and environmental stress. Cooking (1960), for the first time, isolated the protoplasts of plant tissues by using cell wall degrading enzymes, viz., cellulase, hemicellulase, pectinase and protease extracted from a saprophytic fungus *Trichoderma viride*. Later on protoplasts were cultured in vitro.

**Protoplast culture and regeneration** - The protoplasts regenerate a cell wall, undergo cell division and form callus. The callus can also be subcultured. Embryogenesis begins from callus when it is placed on a nutrient medium lacking mannitol and auxin. The embryo develops into seedlings and finally mature plants.

**Somatic hybridisation** - Fusion between protoplasts of the elected parents is induced by a solution of polyethylene glycol (PEG), or by very brief high voltage electric current. Somatic hybridisation allows the production of hybrids between lines and species, that cannot be produced normally by means of sexual hybridisation.

Fusion of cytoplasm of two protoplasts results in coalescence of cytoplasm. The nuclei of two protoplasts may or may not fuse together even after fusion of cytoplasmics. The binucleate cells are known as heterokaryon. When nuclei are fused the cells are known as hybrid, and when only cytoplasms fuse and genetic information from one of the two nuclei is lost is known as cybrid, i.e., cytoplasmic hybrid.

However, production of cybrids which contain the mixture of cytoplasms but only one nuclear genome can help in transfer of cytoplasmic genetic information from one plant to another.
However, production of cybrids can be applicable in plant breeding experiments. For example, in China, cybrid technology in rice is a great success. Such plants are very useful in producing hybrid seeds without emasculation. Today, cybrid technology has successfully been applied to mustard, citrus, tobacco and sugar beet.

Tissue and protoplast cultures have been used in genetic engineering for the transfer of DNA and extrachromosomal bodies -- plasmids mitochondria, chloroplasts. nif(nitrogen fixing) genes from the nitrogen fixing bacterium *Klebsiella pneumoniae* to a strain of the colon bacterium *Escherichia coli*. Isolated protoplasts have great advantage in all the aforementioned uses. For transformation purpose cultured apical meristems are also usable because these can easily be regenerated into whole plants and also because intact DNA taken up by plants appears to be rapidly transported to meristematic regions, where growth and differentiation are centered.