COURSE: MSc Part -1

PAPER – 8

TOPIC- Anatomy and Embryology

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Topic-1

Types of Meristems

The adult body of vascular plants is the result of meristematic activity. Plant meristems are centers of mitotic cell division, and are composed of a group of undifferentiated self-renewing stem cells from which most plant structures arise. Meristematic cells are also responsible for keeping the plant growing. The Shoot Apical Meristem (SAM) gives rise to organs like the leaves and flowers, while the Root Apical Meristem (RAM) provides the meristematic cells for the future root growth. The cells of the shoot and root apical meristems divide rapidly and are considered to be indeterminate, which means that they do not possess any defined end fate. In that sense, the meristematic cells are frequently compared to the stem cells in animals, which have an analogous behavior and function. Meristematic tissue can be classified on the following basis:

1. Classification based on origin and development:

2. Classification on basis of position:

3. Classification on basis of function:

1. Classification based on origin and development:

Meristems on the basis of origin can be classified into following types

(i) Promeristem

A group of young meristematic cells of a growing organ which is likely to develop into a new tissue is Promeristem. It is located in the embryonic tissue and hence are also called as embryonic meristem.In a plant, it occupies a small area at the tip of stem and root. It further divides to form primary meristem

(ii) Primary meristem:

These are derived from promeristem. They are present below the promeristem at shoot and root apices. These cells divide and form permanent tissues.

(iii) Secondary meristem:

With lapse of time and pace of growth a plant attains a structure and a height. In order to stand the the effect of blowing wind and running water it must acquire a girth .Tissues developing from Primary meristem which is responsible for secondary growth is called as secondary meristem. Such as Cork-cambium, cambium of roots and inter fascicular cambium of stem.

2. Classification on basis of position:

On the basis of their position in the plant body, meristems are of three types

(i) Apical meristem:

These are found at the apices or growing points of root and shoot and bring about increase in length. It includes both pro-meristem as well as primary meristem.

Several theories have been put forward to explain the activity of apical meristem:

Apical cell theory:

The theory was first proposed by Hofmeister (1857) and advanced by Nageli (1878). According to this theory, a single apical cell is the structural and functional unit of apical meristem which governs the entire process of apical growth. However, such organization has been found only in cryptogams.

Histogen cell theory:

This theory was given by Hanstein (1868). According to this theory root and shoot apices consists of the central or inner mass called Plerome surrounded by the middle region composed of isodiametric cells called periblem and the outermost uniseriate layer of Dermatogen. Dermatogen gives rise to epidermis, periblem to cortex and endodermis and plerome to vascular bundle and pith. These three layers were called Histogen by Hanstein.

Tunica-corpus theory:

This theory was proposed by Schmidt (1924). According to this theory, mass of dividing cells are of two types: Tunica, the outer consisting of one position of different meristems or more peripheral layers of cells, forming the outer region and Corpus, the central undifferentiated multilayered mass of cell. Epidermis is derived from outer layer of tunica and other tissues from remaining layer of tunica and corpus.

(**ii) Intercalary meristem**:

It lies between the region of permanent tissues and is considered| as a part of primary meristem which has become detached due to formation of intermediate permanent tissues. It is found either at the base of leaf e.g. Pinus or at the base of internodes e.g. grasses.

(iii) Lateral Meristem:

These are arranged parallel to the sides of origin and normally divide periclinally or radially and give rise to secondary permanent tissues. These increase the thickness of the plant part.

III. Classification on basis of function:

On the basis of their function, meristems have been classified into three types:

(i) Protoderm meristem:

It is the outermost layer of the young growing region which develops to form epidermal tissue system.

(ii) Procambium meristem:

It is composed of narrow, elongated, prosenchymatous, meristematic cells that give rise to the vascular tissues system.

(iii) Ground Meristen:

It is composed of large, thick-walled cells which develop to for ground tissue system, i.e. hypodermis, cortex and pith.

IV. Classification on basis of plane of divisions:

The growth pattern and plane of division of meristematic tissue is important to govern the mode of growth.

These tissues can be divided into three types:

(i) Mass meristem:

In such meristem, cell divisions occur in all planes resulting in an increase in volume. It can be observed in meristems of cortex and pith.

(ii) Rib or file meristem:

The cells divide only on one plane e.g., formation of filaments in algae.

(iii) Plate meristem:

These cells divide in two planes resulting to an increase in the area of an organ e.g. Leaf formation.

Topic-2

Apical Meristem

Apical Meristem Definition

The apical meristem is the growth region in plants found within the root tips and the tips of the new shoots and leaves. Apical meristem is one of three types of meristem, or tissue which can differentiate into different cell types. Meristem is the tissue in which growth occurs in plants. Apical is a description of growth occurring at the tips of the plant, both top and bottom. Intercalary meristem is found between branches, while lateral meristem grow in girth, such as in woody plants.

Apical meristem is crucial in extending both access to nutrients and water via the roots and access to light energy via the leaves. Plants must expand in both of these directions in order to be successful. Some plants show apical dominance, in which only one main shoot apical meristem is the most prominent. In plants like this, there is a single main trunk which reaches to great heights. If the apical meristem is cut off, the branches below will start to assume the role of primary apical meristem, which will lead to a bushier plant. Horticulturalists use this phenomenon to increase the bushiness and yield of certain agricultural crops and ornamental plants.

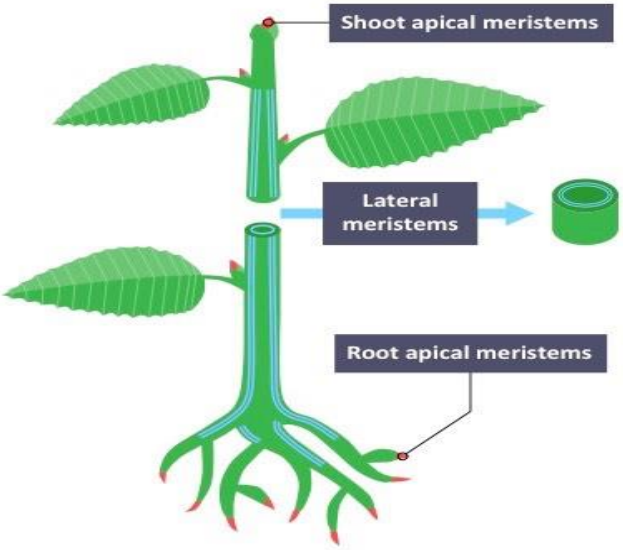
Apical Meristem Function

The apical meristem, found just below the surface of the branches and roots furthest from the center of the plant, is continually dividing. Some cells divide into more meristematic cells, while other cells divide and differentiate into structural or vascular cells. There are two apical meristem locations in most plants. The shoot apical meristem is found in the tips of plants. This apical meristem is responsible for creating cells and growth to drive the plant into the light and air, where it can photosynthesize and exchange built up gases.

The root apical meristem is found at the tips of roots. Sensing the conditions of the soil around the root, signals are created within the apical meristem which direct the plant towards water and desired nutrients. It is for this reason that roots often invade pipes for water and drainage, which carry many of the nutrients they need. The apical meristem, protected by the root cap continues to produce cells even as the root cap is scraped away as it pushes through the dirt. The apical meristem must produce enough cells to not only extend into the soil, but also to replace the cells lost to abrasion.

Apical Meristem Structure

The apical meristem is located just below the root cap in the roots, as seen in the image below. The actual apical meristem is a cluster of densely packed and undifferentiated cells. From these cells will come all of the various cell structure the plant uses. An undifferentiated apical meristem cell will divide again and again, slowly becoming a specialized cell.



Apical Meristems

Causes **primary** growth
(i.e. lengthening of plant)

Occurs at tips of shoots and roots

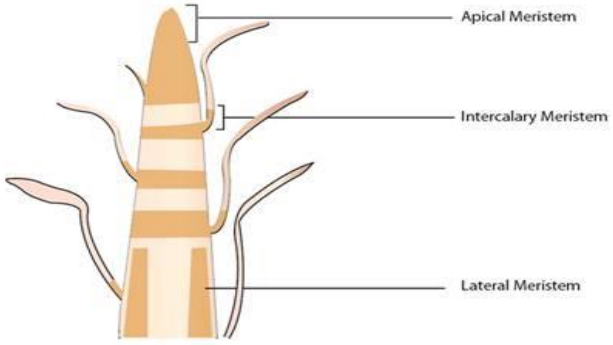
Produces new leaves and flowers

Lateral Meristems

Causes **secondary** growth
(i.e. widening of plant)

Occurs at the cambium

Produces bark on trees



Apical Meristem

In the root apical meristem, the cells are produced in two directions. In the shoot apical meristem, cells are only created in one direction. The shoot apical meristem may exist at the tips of plants, as in many dicots, or may start slightly below the soil and generate leaves which grow upward, like most monocots. However, in both groups the shoot apical meristem is the growth center of all above ground growth.

Interestingly, the shoot apical meristem in most plants is capable of producing an entire plant, whereas the root apical meristem cannot. Scientists have used the ability of the shoot apical meristem to clone many species of plant. By simply cutting off the apical meristem and transferring it to an appropriate growth medium, the apical meristem will develop roots and differentiate into a whole new plant. As an added benefit, more apical meristems form on the plant, and can be harvested for more clones. In this way, a desirable plant can be replicated almost indefinitely.

Topic-3

Anomalous secondary growth

In many vascular plants, secondary growth is the result of the activity of the two lateral meristems, the cork cambium and vascular cambium. Arising from lateral meristems, secondary growth increases the girth of the plant root or stem, rather than its length.During secondary growth Cambium becomes active as a meristematic tissue and cuts of new cells called as Secondary phloem(towards otside) and secondary xylem(towards inside). In this manner the plant goes on adding cells to provide girth to the plant. The behavior of the cambium remains same throughout the growth. However, in Anomalous secondary growth" cambial conformations, cambial products, and cambial numbers differ from the most common "normal" condition, namely, a single cylindrical cambium that produces phloem externally and xylem internally. The term "variants" is employed here as a way of referring to the less common types; "anomalous" may give the misleading impression of a disorderly action.

The following points highlight the five major reasons of anomalous secondary growth in plants. The reasons are:

1. The Activity of Normal Cambium is Abnormal

2. Abnormally Situated Cambium Forms Normal Secondary Vascular Tissues

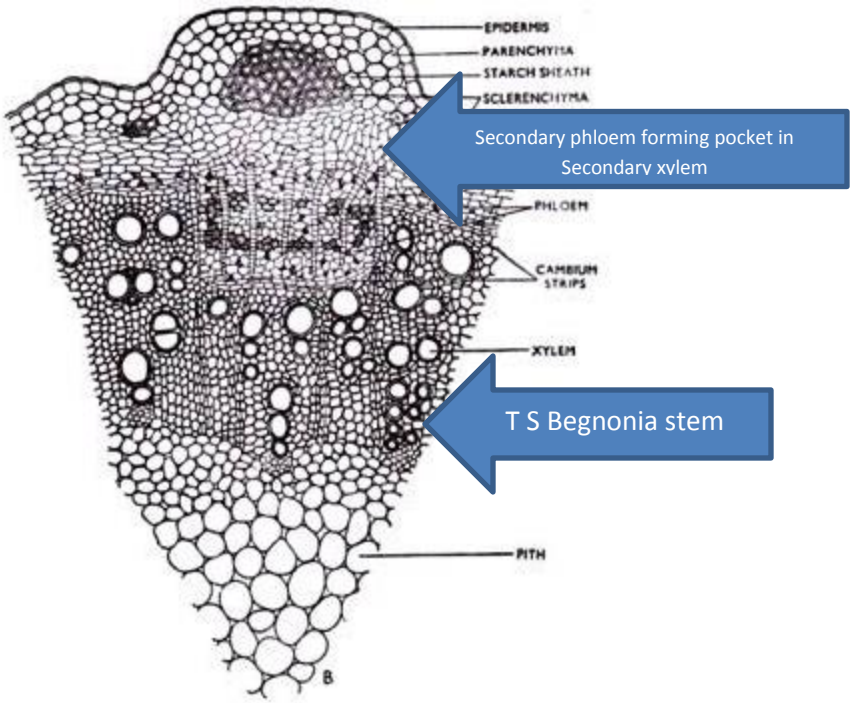
3. Formation of Secondary Tissues by Accessory Cambium

4. Formation of Interxylary Phloem

5. Formation of Intra-Xylary Phloem.

1. **The Activity of Normal Cambium is Abnormal**:

Cambia of this category function mostly in two ways. Certain segments of cambia cease producing secondary xylem; instead these segments donate secondary phloem only towards exterior. The other segments of cambium produce secondary phloem and secondary xylem normally. As a result a ridged and furrowed stele is formed (ex. Bignonia). In other cases the interfascicular cambium forms non-vascular tissues. Vascular- tissue formation is restricted to fascicular cambium only (ex. *Aristolochia, Tinospora*, *Clematis* etc.).

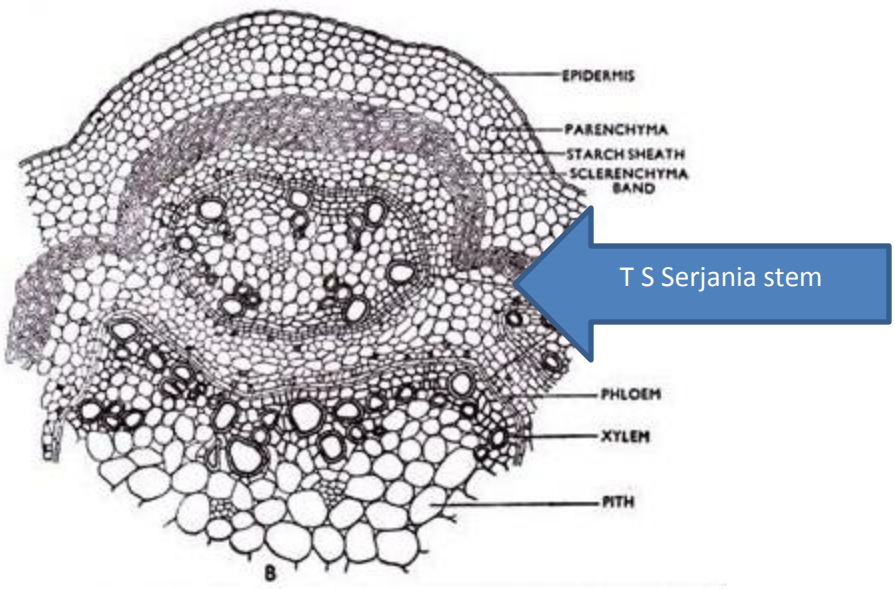


2 Abnormally Situated Cambium Forms Normal Secondary Vascular Tissues:

Cambia of this category produce secondary vascular tissues in normal fashion, but their positions are anomalous. These cambia form discrete vascular cylinders and their arrangements differ according to species. Most of the climbing species of Serjania exhibit a principal vascular cylinder surrounded by small peripheral vascular cylinders. The vascular cylinders and the pericycle compose the stele. Endodermis delimits the stele on the peripheral side.

In Serjania stem several cambial layers are present from the first. In the cross- section of stem the cambia are arranged in various ways. Different species of Serjania exhibit the various arrangements of cambia. In the stele of Serjania caracasana stem a central principal cambial cylinder occurs.

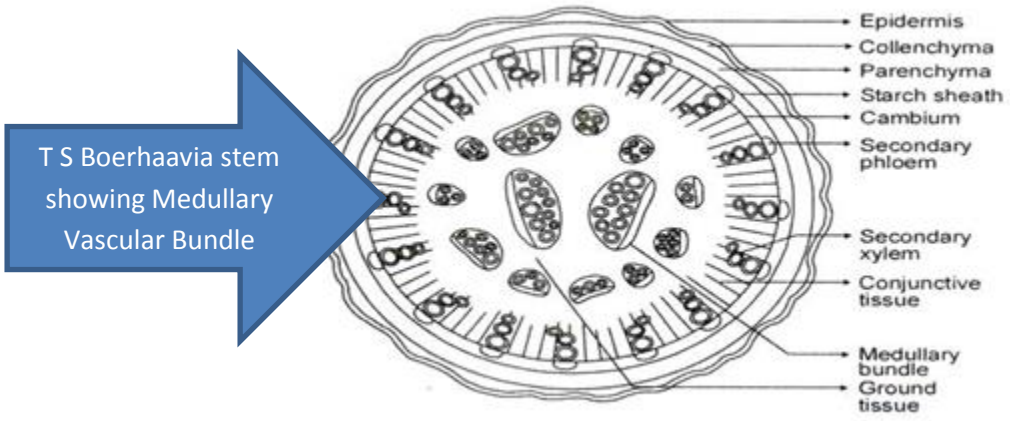
Several minor cambial cylinders surround the principal cambial cylinder. In Serjania corrugata there is no central cambium. In the stele five to seven peripheral cambial cylinders occur in a circle and the cambia are approximately equal. In all species individual cambial layer behaves in normal fashion, i.e. donates secondary xylem on the inside and secondary phloem on the peripheral side.



3. Formation of Secondary Tissues by Accessory Cambium:

Baugainvillea, Amaranthus, Boerhaavia, Achyranthes, Celosia etc. exhibit accessory cambia that form vascular and non-vascular tissues. This type of anomaly is illustrated below taking the example of *Boerhaavia diffusa*.

In *Boerhaavia diffusa* endodermis delimits stele of stem. All segments of endodermis are not always distinct. Just below the endodermis there occurs the pericycle. It is narrow and consists of one or two cell layers. In older stems just below the endodermis a few scattered fibers are present.The position of fibers is of special interest because they locate the endodermis when the latter is indistinct. The other structures of stele include parenchymatous ground tissue, conjunctive tissues and primary vascular bundles.



4. Formation of Interxylary Phloem

The secondary phloem strands or layers that remain surrounded by secondary xylem are referred to as interxylary phloem. Interxylary phloem is also termed as included phloem/interxylary soft bast as it remains embedded in wood. Eames and MacDaniels illustrated the following two methods by means of which interxylary phloem becomes embedded in secondary xylem.

In Combretum, Leptadenia and Entada, during secondary growth a normal cambium ring is formed by the union of intra-fascicular and interfascicular cambium. The cambial ring functions normally, i.e. produces secondary xylem inside and secondary phloem outside.

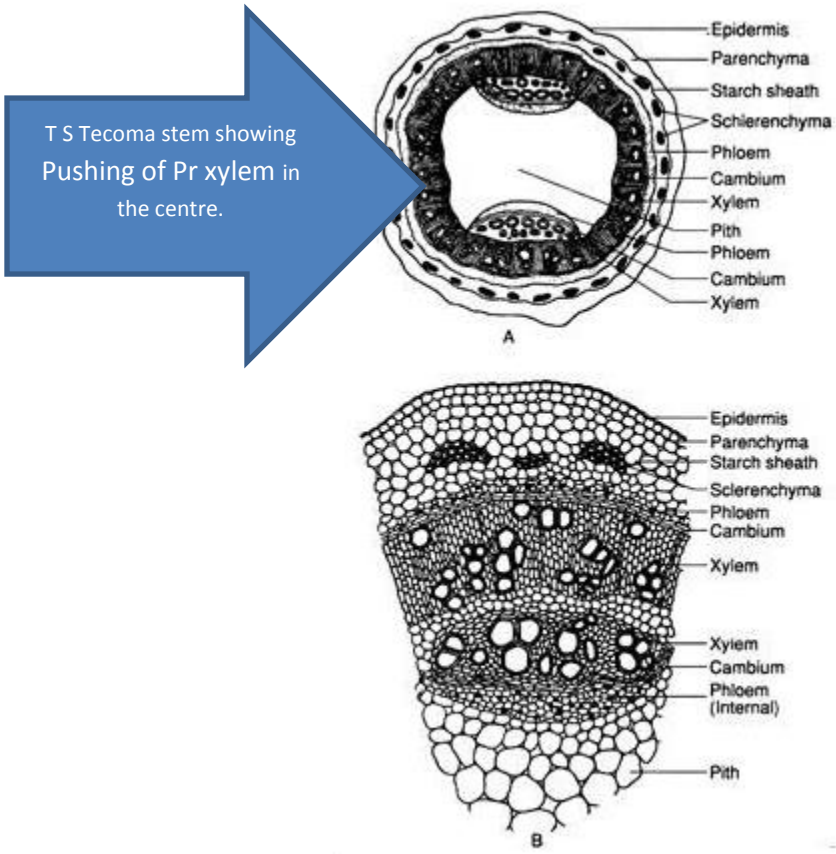
Subsequently certain small segments of cambial ring donate secondary phloem toward the inside for a brief period. Normally these segments produce secondary xylem. During the production of interxylary soft bast these segments form secondary phloem in place of secondary xylem.

5.Formation of Intra-Xylary Phloem (= internal phloem): .

The stele of *Tecoma radicans* is siphonostele. The primary vascular bundles are arranged more or less in a ring and each vascular bundle is collateral, open with protoxylem endarch. During secondary growth intra-fascicular cambium and interfascicular cambium unite to form complete cambium ring. The cambium ring has normal function, i.e. produces secondary phloem on the peripheral side and secondary xylem towards inner side, the secondary xylem being in excess of secondary phloem.

As a result primary vascular bundles are pushed towards the centre. After a period of activity two strips of additional cambia originate below secondary xylem on two opposite sides of pith. Each

strip of cambium functions abnormally. Each cambial strip donates secondary phloem towards the centre or pith side and secondary xylem towards the peripheral side.



Anomalous secondary growth in Dracaena stem:

Dracaena is arborescent in habit, and belongs to the monocotyledonous family Agavaceae. The vascular bundles of monocotyledons are closed, i.e. intra-fascicular cambium is absent. So monocotyledons lack normal secondary growth from a vascular cambium. In Dracaena the stele is atactostele.

The primary vascular bundles are distributed over the ground tissue without having any definite arrangement. The primary vascular bundles are not compactly arranged on the ground tissue and the interfascicular region is moderately wide. The ground tissue is composed of parenchyma cells and the parenchyma shows no radial seriation of cells.

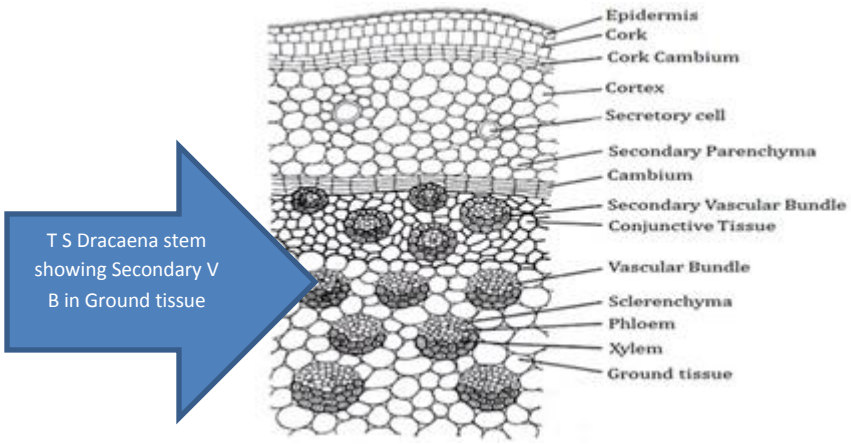
Each primary vascular bundle is leptocentric/amphivasal, i.e. the xylem completely surrounds phloem and there exists no intra-fascicular cambium. Each vascular bundle is circular or oval in

transverse section. The xylem consists of tracheids only. Protoxylem with annular and spiral thickening is present.

The cross-section of mature stem of *Dracaena*, where certain amount of secondary growth has occurred, exhibits secondary vascular bundles and ground tissue commonly termed as conjunctive tissue. Conjunctive tissues are parenchymatous, the walls of which are thin and may sometimes become thickened or even lignified.

The conjunctive tissue exhibits radial arrangement of cells and thus aids in differentiation from the primary interfascicular ground tissue. The secondary vascular bundles are to some extent arranged in radial rows. The vascular bundles are more or less compactly arranged and anastomose in some regions in contrast to primary vascular bundles.

Each vascular bundle is oval in t. s. and leptocentirc/amphivasal like primary vascular bundles. The secondary phloem is small in amount in comparison to primary phloem. The secondary phloem elements are sieve tubes, companion cells and phloem parenchyma. The sieve tubes are short with transverse end walls and the sieve plate is simple



Students are advised to prepare notes also on

1Cork cambium

2 Periderm

3.Types of tissues

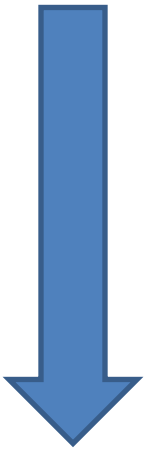
4. Bark

5.Healing of wound.

6.Guard cells and its function

7. Lenticel

Embryology



Topic-4

Protocol for Tissue Culture

Protocols for Plant Tissue Culture

Tissue culture involves several stages of plant growth, callus formation, shooting, roots, and flowering. It starts with sterilizing a piece of plant tissue.

Sterilizing Plant Materials

Inside the glove box, +all plant material is sterilized in diluted domestic bleach solution containing 1 drop of detergent as a surfactant.Plant pieces is put in a jar containing the bleach for 10–20 minutes. Agitated Frequently. The tissue is transferred in the bleach solution to the sterile area. The chlorine solution is then discarded. Plant pieces is then rinsed twice with sterile water.

Callus Formation Protocol

1.Basic media is prepared without added coconut milk or malt extract.

2. The sterilization steps for the medium, instruments, and chamber is strictly followed.

3.The cultures, sterile media, sterilized tools, and sterile paper towels, and sterile water are placed at one end of the chamber. At the other end of the chamber, organize a small paper bag for trash, a jar of bleach solution, and a spot for freshly inoculated cultures..

4. Plant tissue is sterilized, following this protocol.

A sterilized piece of tissue is taken from the jar with a pair of forceps (do not touch the plant material with your hands). Working on a dampened sterile paper towel, use the scalpel or a razor blade to cut the tissue sample into 2- to 3-cm-long pieces.

One piece of the tissue is then placed into each container (it is important to have only 1 shoot per container at this stage so that if the shoot is contaminated it cannot spread to the others). Shut the lids of the containers. Store jars at room temperature away from direct sunlight. Callus will start to become visible after about 7 days post-initiation.

Friable type II callus should be visually selected at each subsequent transfer to maintain an optimal phenotype. Callus should be transferred to fresh medium at 2- to 4-week intervals, depending on growth rate. Switching to different types of media also helps maintain vigor.

Callus can be maintained at room temperature in the dark.

Shoot Multiplication Protocol

Prepare a batch of media containing 5% to 10% coconut milk. Follow the sterilization steps for the medium, instruments, and chamber.With a pair of forceps, remove a culture from its container. Moisten the sterile paper with some sterile water. It is important to do all the manipulation on a damp paper towel, as these plants are very soft and can desiccate readily. The culture is then sliced and transfered to new jars. At this stage, up to 5 stems may be put inside each jar.Store cultures as explained in the previous stage.

Root Formation Protocol

Once enough shoots have been established, let them grow to at least 2 cm before beginning the rooting process.Follow the sterilization steps for the medium, instruments, and chamber..

With a pair of forceps, remove a stem from its container. Wash all of the media off the culture and transfer to malt media. (Malt contains auxins, which promote root formation.)

Up to 5 shoots may be put in each culture vessel. Containers are stored in their usual place as before. Roots should form within 2 to 4 weeks.

Acclimatization Protocol

Fill plastic “vegetable” bags with a potting mix that contains no fertilizer. Autoclave for 15 minutes in the pressure cooker when cool, inoculate with mycorrhizae. Remove the rooted plants from agar medium using a pair of forceps. Wash off the agar thoroughly from the roots using lukewarm water. Poke a hole in the middle of the potting mix, using a sterile instrument; gently insert the roots in that hole. Dampen the potting mix with basic nutrient mix. Spray the foliage with a hand spray containing sterile water. Keep these bags inside larger plastic containers with a glass cover, out of direct sunlight. Gradually remove the glass cover, but watch for signs of desiccation and if needed, use the hand spray to spray water on the foliage. Gradually increase the light intensity for the plants also.When the roots are well established and the plants are acclimatized (this should take about 4–6 weeks), they can be given fertilizer and be treated like any other plant.

Plant Protoplast Preparation Protocol

Perform all of the following in the glove box.

Buffer Solution. Dissolve 56.94 g of mannitol in 200 mL of distilled water. Add distilled water to bring the final volume to 500 mL.

Digestion Solution. Measure 10 mL of buffer solution into a 15-mL test tube or small beaker. Measure 0.1 g pectinase and 0.2 g cellulace onto weighing paper. Drop the premeasured,

powdered enzymes into this solution. Swirl the beaker or cap the test tube and shake it back and forth until the enzymes are completely dissolved.Carefully pour all 10 mL of enzyme solution into the bottom of a sterile jar.Use forceps to float each tissue sample on the surface of the enzyme solution.Seal the jar with Saran Wrap or tape and leave it at room temperature (approximately 25°C) overnight. If proper equipment is available, gentle agitation of the dishes will be helpful.The next day, gently swirl and shake the solution in the petri dish. (If no protoplasts are observed with the microscope, let the solution stand for another 15–30 minutes, then look again for protoplasts.).At the end of the digestion period, gently shake the petri dish to release the protoplasts.Filter the enzyme-protoplast suspension through successively smaller filters, starting with the 100-mm sieve. One sieve will trap mostly protoplasts.

Topic-5

Development of megaspore in Angiosperm

Megasporogenesis

During megasporogenesis, the diploid megaspore mother cell undergoes meiosis and gives rise to four haploid nuclei. Angiosperms exhibit three main patterns of megasporogenesis, referred to as monosporic, bisporic, and tetrasporic. These three patterns are summarized in Figure given below. The three types differ mainly in whether cell plate formation occurs after these divisions, thus determining the number of meiotic products that contribute to the formation of the mature female gametophyte.

In the monosporic pattern, both meiotic divisions are accompanied by cell plate formation, resulting in four one-nucleate megaspores. Subsequently, three megaspores, generally the micropylar-most megaspores, undergo cell death.

Monosporic megasporogenesis can be of two types:

a) Monospric *Polygonum*

b) Monosporic *Oenothera*

In the bisporic pattern, cell plates form after meiosis I but not meiosis II. The result is two two-nucleate megaspores, one of which degenerates.

Bisporic Megasporogenesis can be of the following types:

a) Bisporic Allium type

In the tetrasporic pattern, cell plates fail to form after both meiotic divisions, resulting in one four-nucleate megaspore. Thus, these three patterns give rise to a single functional megaspore that contains one (monosporic), two (bisporic), or four (tetrasporic) meiotic nuclei. The monosporic pattern is the most common form and is represented within the Polygonum pattern (Maheshwari, 1950; Willemse and van Went, 1984; Haig, 1990; Huang and Russell, 1992).

Tetrasporic megasporogenesis are of the following types:

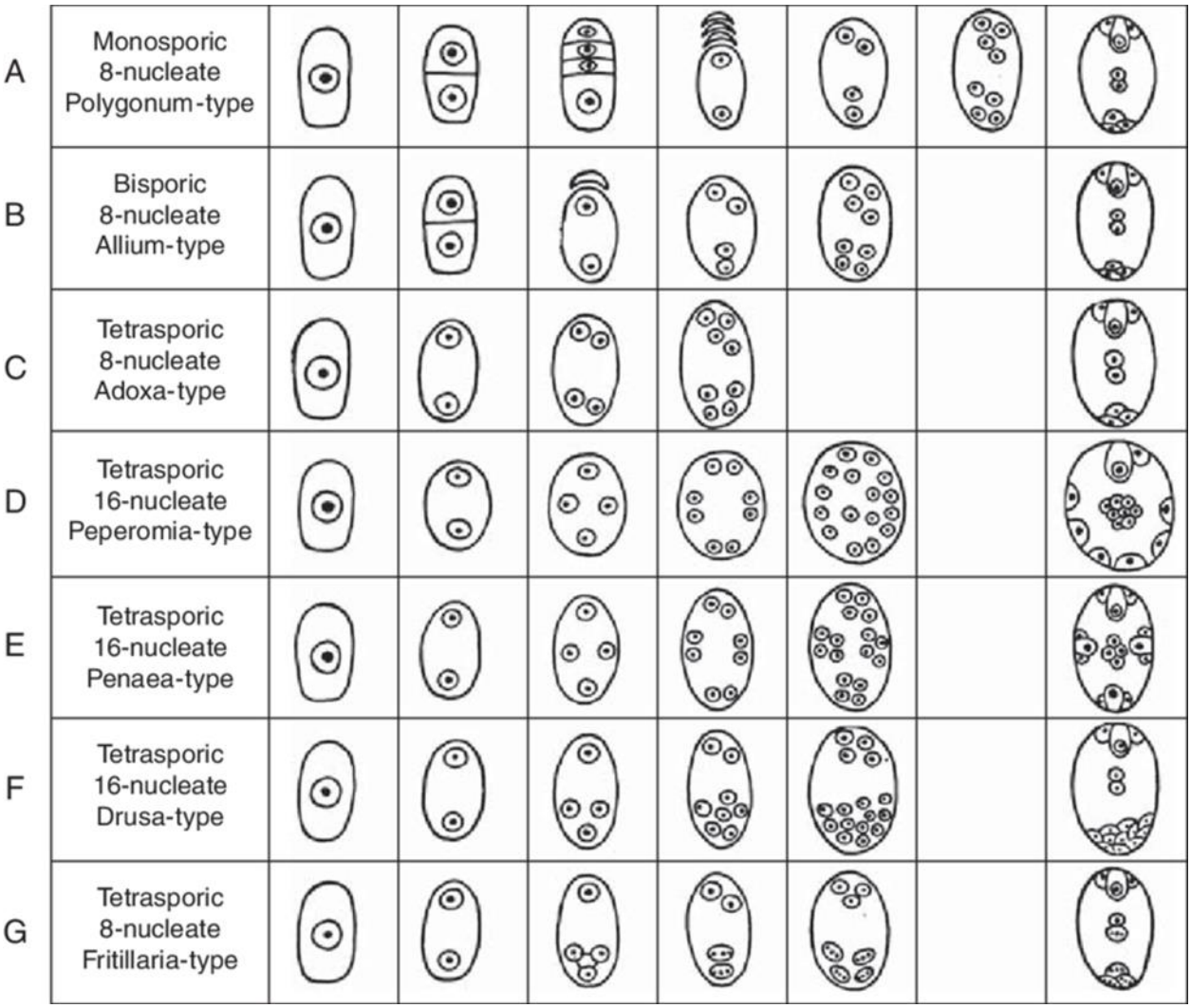
a) Adoxa type

b) Pepromia type

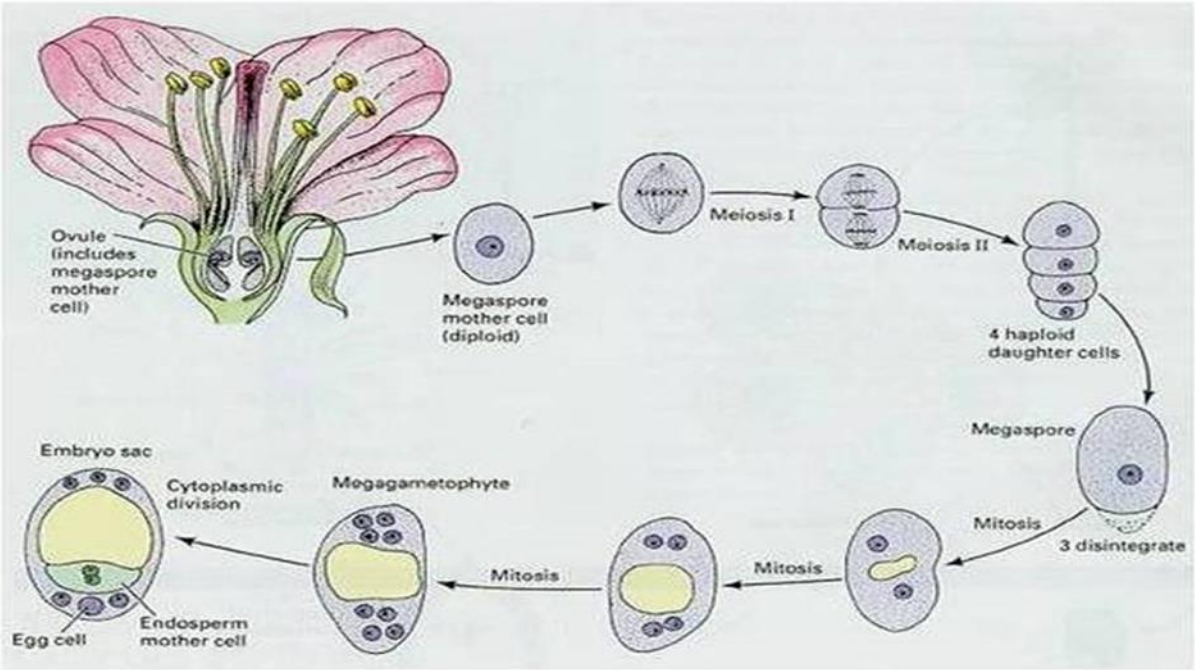
c) Penaea type

d) Drusa type

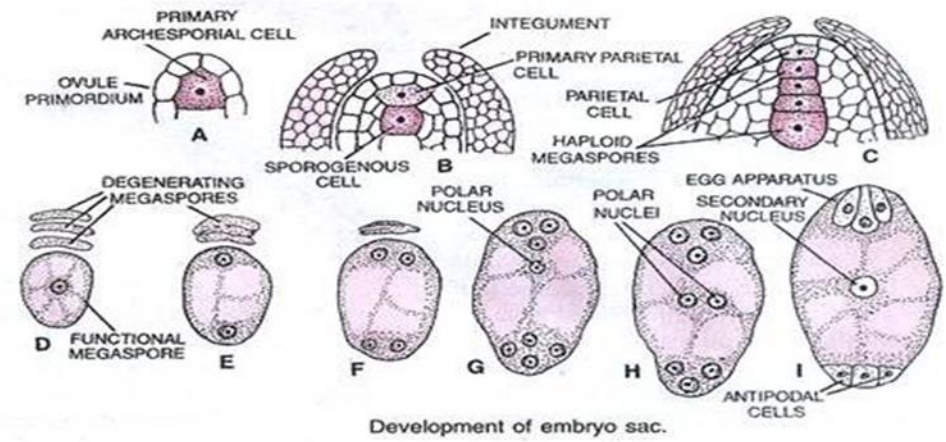
e) Fritillaria type



Megasporogenesis in Angiosperm(Left side)



Megasporogenesis in Angiosperm



Development of functional Megaspore into Embryo

Topic-6

Development of Embryo sac in Angiosperm

Monosporic, Normal or Polygonum Type

It is commonly found in plant. It is commonly called normal type. However, it was first clearly described in Polygonum. Therefore, it is also called as Polygonum type.

This embryo sac has four well-defined megaspores. One of which gives rise to the embryo sac. The functional megaspore enlarges. Its nucleus divides. A large vacuole is formed between the nuclei. Thus the daughter nuclei move to the micropylar and chalazal poles of the embryo sac. Each nucleus divides twice. Thus four nuclei are formed at each pole. One nucleus from each pole migrates to the centre of the embryo sac. The two nuclei fuse to form a diploid secondary nucleus. Three nuclei at micropylar end are surrounded by membranes. They form egg apparatus. The central cell enlarged arid become egg cell. The other two cells becomes synergid. Thus embryo sac is formed containing 8-nucleoli and later 7-celled during its development.

Bisporic or Allium Type

This type of embryo sac is found in Allium. It is found in many monocot and dicot families. Two dyad cells are formed during first meiotic division duri-j. megasporogenesis. One of two dyad cell is abiyied The of the surviving dyad cell towards the chalazal end &lies to ft TM two haploid nuclei. These are called megaspore nuclei. These nuclei move towards opposite ends. These nuclei divide tw ice to form eight nuclei. One nucleus from each pole migrates to the centre of the embryo sac. Three nuclei at the upker end produce egg apparatus. The nuclei present at lower end form antipodal cells. In this way 8- nucleate bisporic embryo sac develops.

Tetrasporie Type

In this type of embryo sac wall is not formed after the meiotic nuclear division. All four haploid megapsore nuclei take part in the formation of the embryo sac. The resultant embryo sac• may be 8- nuceleate or 16-nucleate. Thus it has two types:

a) Plunrnbago Type (8-Nucleate): In this case, the megaspore nuclei arrange themselves in a cross-like manner. One lies at the micropylar ends and the other lies at the chalazal end. The other two are present at each side of the embryo sac. Each nucleus divides once. Thus pairs of four nuclei are formed. One nucleus from each pair migrates to the centre. They fuse to form tetraploid secondary nucleus. The nucleus at micropylar and form the egg cell. The rest three nuclei degenerate. There are no antipodal cells and synergids.

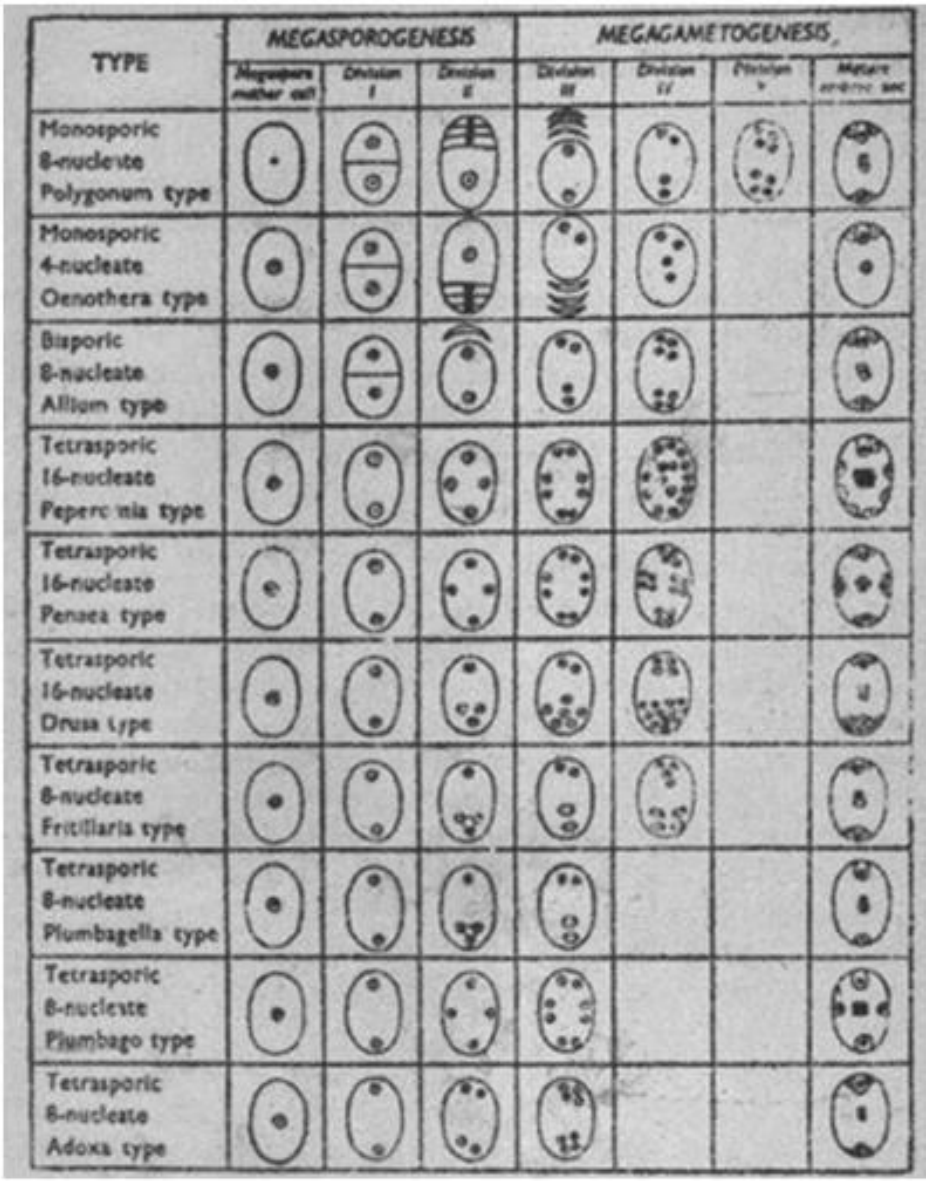
b) Fritillaria Type (8-Nucleate): This type of embryo sac occurs in a large number of genera. In this case, Three out of four megaspore nuclei are arranged in 3 + 1 fashion. Three nuclei migrate to the chalazal end. The remaining nucleus comes at the micropylar pole. The micropylar nucleus divides to form two haploid nuclei. The three chalazal nuclei fuse. The fusion nucleus ‘divides to form two triploid nuclei. Now the embryo sac contains four nuclei, two haploid micropylar nuclei and two triploid chalazal nuclei. Later each nucleus divides. Thus they produce four haploid nuclei at micropylar end and four triploid nuclei at chalazal end. One nucleus from each pole migrates to the centre. These fuse to forms a tetraploid secondary nucleus. The nuclei at micropylar end form egg apparatus. The nucleus at the chalazal end gives rise to antipodal cells.

c) Pen.tea Type (16 Nucleate): In this case, 16 nuclei are arranged in quarters. One is present at each end of the embryo-sac and two are present at the sides. Three nuclei of each quarter become cells. The fourth nuclei of each quarter moves towards the center and act as polar nucleus. Therefore, there are four triads and four polar nuclei. One cell of the micropylar triad is the egg. It is the only functional cell.

d) Drusa Type (16 Nucleate): In this case, one megaspore nucleus moves towards the micropylar. The remaining three megaspore nuclei move towards chalazal end. Each nucleus divides twice. Thus four nuclei are produced at micropylar end and twelve at chalazal end. One nucleus from each migrates towards the centre of the embryo sac. They fuse to form secondary nucleus. The three nuclei at micropylar end form egg apparatus. The eleven nuclei at chalazal end form antipodal cells.

e) Adoxa Type (8-Nucleate): The four haploid megaspore nuclei present in the cytoplasm undergo a mitotic division. They produce eight nuclei. These nuclei are arranged in typical manner. Three of them come at the micropylar end. Three comes at the chalazal end. And two come in the centre (fusion nucleus). Thus normal 8.nucleate seven celled embryo sac is formed.

0 Paperoma tye (16 Nucleate): In this case, each of four megaspores nuclei divides twice. They form 16 nuclei. These are uniformly distributed at the periphery of the embryo sac. Two nuclei at micropylar end form an egg and a Synergid. Eight of them fuse to form secondary nucleus. The remaining three stay at the periphery of the embryo sac.



Types of Embryo sac in Angiosperm

Topic-7

Double Fertilization in Angiosperm

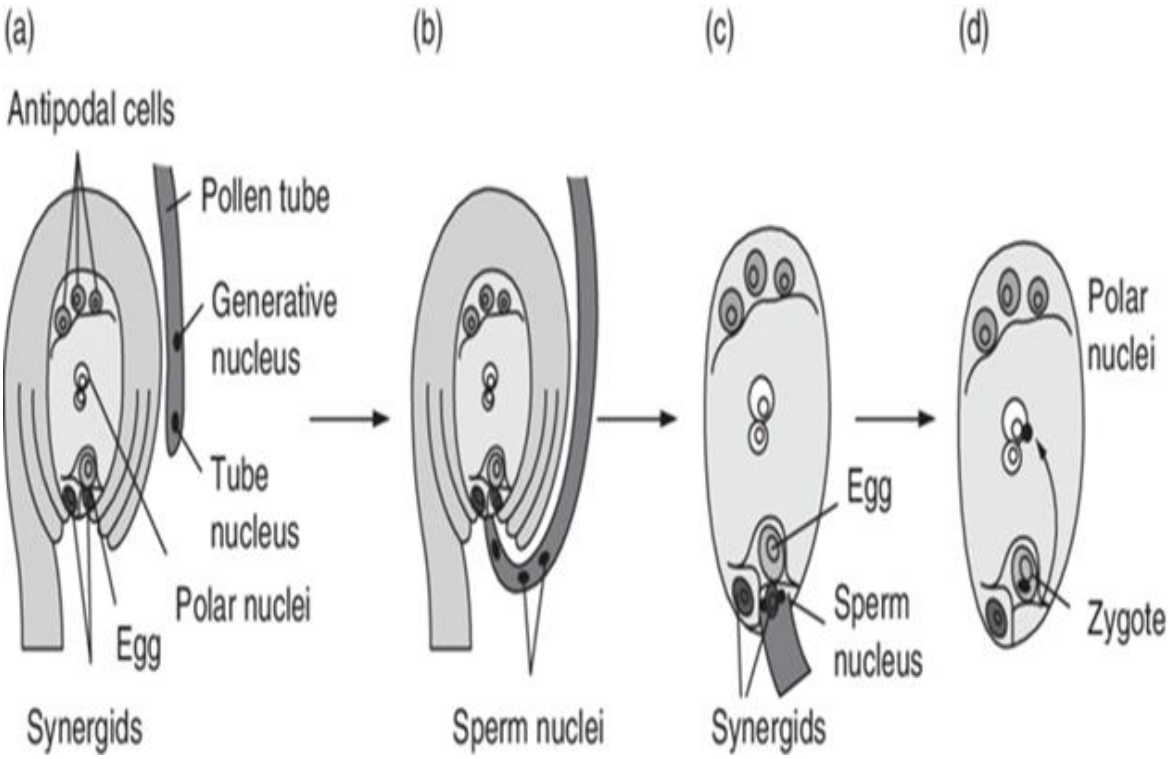
Unambiguous proof of the actual fusion of the male and female gametes embodied in fertilization in angiosperms is traced to a monographic publication of Strasburger (1884). This work was mostly devoted to the nuclear cytology of pollen grains and pollen tubes of plants belonging to a wide range of families and to the fate of male gametes delivered by pollen tubes in the embryo sacs of Gloxinia hybrida (Gesneriaceae), Himantoglossum hircinum, Orchis latifolia (Orchidaceae), and Monotropa hypopitys (Monotropaceae). The most complete, illustrated details were provided on M. hypopitys in which it was shown that one of the two male gametes conveyed by the pollen tube fused with the nucleus of the egg. At that time the male gametes were known as the generative nuclei and it was also uncertain whether these gametes were true cells in their own right or just naked nuclei. However, the observation that a male gamete fused with the egg in the act of fertilization was contrary to a previous puzzling finding that this event was orchestrated by the diffusion of cytoplasmic contents of the pollen tube (Maheshwari, 1950). Although Strasburger's work identified the embryo as the resulting product of fertilization, understanding of the fate of the second male gamete discharged by the pollen tube and the source of origin of the endosperm (albumen) remained as major hurdles in gaining a complete insight into the dynamics of fertilization in angiosperms.

The breakthrough occurred when Nawaschin (1898, 1899) in Russia showed that in ovules of Lilium martagon and Fritillaria tenella (Liliaceae), both male gametes from the pollen tube penetrated the embryo sac; whereas one of them fused with the nucleus of the egg cell, the other fused with the polar fusion nucleus (at that time known as the definitive nucleus) floating in the central cell, initiating a second fertilization event. The results of this work were orally presented on August 24, 1898 to the botanical section of the ‘Naturforscherversammlung’ held in Kiew and were published as an abstract in the following year (Nawaschin, 1899); the full paper, communicated for publication on September 30, 1898, appeared within a few months after the meeting (Nawaschin, 1898). Thus, reverent credit is due to Nawaschin for the discovery of the two fusion events during fertilization in flowering plants

The Process of Double Fertilization

Double fertilization is a major characteristic of flowering plants. In this process, two male gametes fuse with one female gamete wherein one male gamete fertilizes the egg to form a zygote, whereas the other fuses with two polar nuclei to form an endosperm.An ovary contains at least one ovule. Inside an ovule, cells divide to produce an egg and two other cells called polar nuclei. These three cells are haploid, which means they have one set of chromosomes, and is designated by n. Most cells in angiosperms are diploid, or have two sets of chromosomes. In

diploid (2n) cells, one set of chromosomes comes from the male parent and the other set comes from the female parent.In addition to eggs and polar nuclei, sperm cells from a pollen grain are also haploid. Once the pollen tube reaches the micropyle, or the opening of an ovule, it releases two haploid sperm cells into the ovule. One sperm cell will fuse with the egg, resulting in a diploid zygote. The other sperm cell will fuse with the two polar nuclei, creating a triploid (3n) structure that will grow rapidly into the endosperm.: A pollen tube releases two sperm cells into an unfertilized ovule. 2: Double fertilization occurs when one sperm cell fuses with the egg to produce a zygote, and the other sperm cell fuses with the two polar nuclei to make the endosperm. 3: After double fertilization, a seed and fruit develop.



Process of Double Fertilization in Angiosperm

Topic-8

Endosperm

Endosperm is the tissue that surrounds and nourishes the embryo in the seeds of angiosperms (flowering plants). In some seeds the endosperm is completely absorbed at maturity (e.g., pea and bean), and the fleshy food-storing cotyledons nourish the embryo as it germinates. In others, some of the endosperm is present until germination (e.g., wheat, castor bean), and the cotyledons are typically thin and membranous and serve to absorb the stored food from the endosperm upon germination. In the coconut, the liquid endosperm contains important growth substances. Endosperm accounts for the economic importance of cereal grains and oilseed.

The initiation of endosperm is a definitive characteristic of the double fertilization of angiosperms. Its development requires the fusion of at least one polar nucleus in the embryo sac with one of the two sperm nuclei from the pollen grain. In gymnosperms the nutritive material of the seed is present before fertilization.

In gymnosperms it represents the female gametophyte. In angiosperms the endosperm is a special tissue which is formed as a result of vegetative fertilization, triple fusion or fusion of a male gamete with diploid secondary nucleus of the central cell. The fusion product is primary endosperm cell having a triploid endosperm nucleus.

Depending upon the mode of its formation, angiospermic endosperm is of three types—

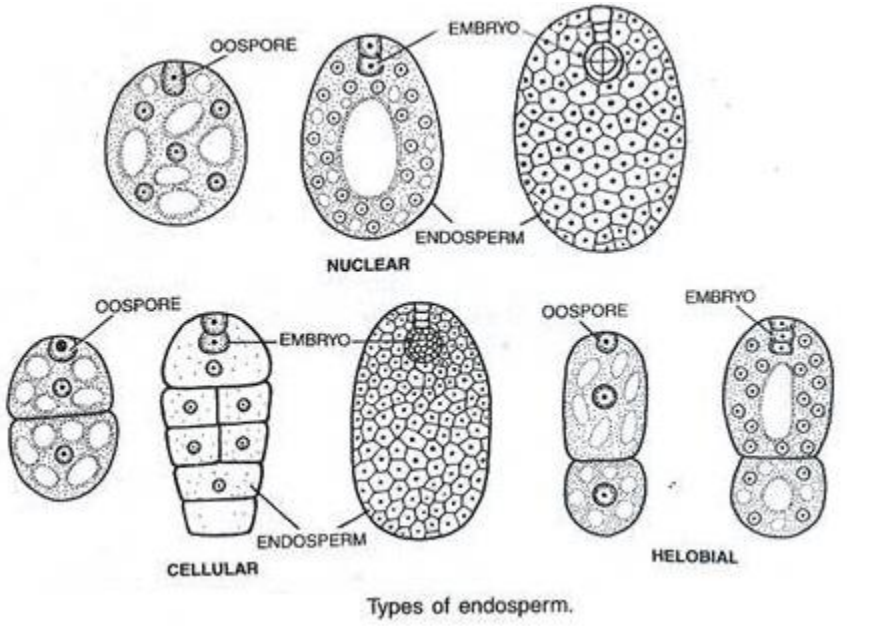
1. Nuclear,

2. Cellular and

3. Helobial.

1. Nuclear Endosperm

The primary endosperm nucleus divides repeatedly without wall formation to produce a large number of free nuclei. Meanwhile central vacuole appears in the central cell and pushes the cytoplasm containing the nuclei to the periphery. The cytoplasm thickens so that the vacuole decreases in size.It ultimately disappears with the exception of a few cases. The multinucleate cytoplasm undergoes cleavage and gives rise to a multicellular tissue, e.g., Maize, Wheat, Rice, Sunflower, Capsella bursa-pastoris. In several cases, the cell wall formation remains incomplete.For example, Coconut has mul-ticellular endosperm (called coconut meal) in the outer part and free nuclear as well as vacuolated endosperm (called coconut milk) in the centre. In Pharsalus wall formation occurs around the embryo only while in Crotalaria it is restricted to the upper half.



Types of endosperm.

Types of Endosperm found in Angiosperm

2. Cellular Endosperm

Every division of the primary endosperm nucleus is followed by cytokinesis. Therefore, endosperm becomes cellular from the very beginning, e.g., Bal-sam, Datura, Petunia.

3. Helobial Endosperm:

It occurs in order helobiales of monocots. The endosperm is of intermediate type between cellular and nuclear types. The first division of primary endosperm nucleus is followed by transverse cytokinesis to form two unequal cells, larger micropylar and smaller chalazal. Micropylar cell grows faster than the chalazal one.

Further development in both the cells occurs like that of nuclear endosperm, i.e., multinucleate stage followed by wall formation, e.g., Asphodelus. However, chalazal chamber often remains smaller and may degenerate.

Functions of Endosperm

The tissue of young endosperm is rich in food materials and various growth hormones.

It regulates the precise mode of embryo development and nourishes the developing

embryo. During seed germination, the reserve food materials stored in mature endosperm

are digested and utilized for the growth of the seedling until the later develops chlorophyll and is able to manufacture its own food. In some plants, the seed coat and the fruit wall are consumed by the endosperm, which ultimately becomes exposed to sunlight and develops chlorophyll for photosynthesis. Rarely the outermost layer of such exposed endosperm takes on a protective function. In the absence of endosperm, the embryo usually aborts.

Endosperm development is a characteristic feature of all the families of angiosperms with the exceptions of Podostemaceae and Trapaceae. In the Orchidaceae endosperm degenerates quite early. Endosperm may be used up by the embryo as such the mature seed has no traces of it (exalbuminous seed). In most monocotyledons it persists (albuminous conditions). In the main food plants such as wheat, rice, maize and sorghum it is the starchy endosperm that forms the bulk of the grain. In many legumes the mature seed has food reserves in the cotyledons rather than in the endosperm. In the castor seed endosperm is laden with fatty substances.

The cereal endosperm is made of very different tissues at maturity. The outer aleurone layer consists of living cells. The endosperm usually occupies the bulk (87%) of the grain and about 10% of the endosperm dry weight is aleurone. The aleurone layer stores lipid (about 90% of total endosperm lipid) and also contain 20% of protein. During germination, hydrolytic enzymes are produced in the aleurone layer and these are released into starchy endosperm where the reserves are hydrolysed.

When the barley grains are soaked in water gibberellins (GA, and GA,) are released from scutellum of embryo and diffuses into the endosperm. The largest tissue for this hormone is the aleurone, which responds by breaking down its own protein reserves and by secreting enzymes (mostly hydrolytic) into the starchy endosperm.

Some of these enzymes are newly synthesized (e.g. a-amylase) and some are (e.g. P-glucanase) pre-existent Probably all cereals except sorghum, have aleurone that responds similarly.

Thus endosperm has a very important role in the development of the embryo. In most of inter varietal and interspecific crosses, embryos fail to form because of failure of endosperm formation.

VARIANTS OF ENDOSPERM

The mode of development of endosperm discussed above conforms to one of the three basic types: Nuclear, Cellular and Helobial. However, variations arise at a later stage of development, such as composite endosperm, ruminate endosperm and mosaic endosperm.

Composite Endosperm

In the family Loranthaceae, the development of endosperm is unique. The ovary lacks ovules. The sporogenous tissue located at the base of ovary develops several embj.0 sacs whicb elongate considerably, some of them even entering the style.

After fertilization, the primary endosperm nucleus of eacb embryo sac moves to the basal part where it divides to form cellular endosperm . During fimher development, the endospenns of all the embryo sacs in an ovary enlarge and become fused to produce a composite endospm mass . Several proembryos belonging to individual embryo sacs with long suspensors develop but only one survives and attains maturity. .

Ruminate Endosperm

In certain plants the surface of the mature cellular endosperm shows a high degree of irregularity and unevenness, giving a ruminated appearance (rumination means as if chewed). It is caused either by the activity of the seed coat or by the endosperm itself. Ruminate endosperm is found in abo~t 32 families of Angiosperms. On morphological

basis, Periasamy (1962) distinguishes seven types: Annona, Passiflora, Myristica Spigelia, Verbascum and Coccoloba and Elytrariu. In all these types except Elytraria irregularities occur in the growth of integuments which bring about the rumination of endosperm. In Elytraria during the development of seed, localized regions in the peripheral layers of cellular endosperm show active growth causing rumination.

Mosaic Endosperm

In some plants patches of two different colours appear in the tissues of the endosperm providing a mosaic design. In maize, red and white patches of tissues are sometimes seen in the grain. The occurrence of such endosperm has also been reported in Petunia, Lycopersicon and Acorus etc. Several theories have been advanced to explain the development of mosaic endosperm but none of these has been cytologically demonstrated. The most appealing explanation for the development of such endosperm is said to be the aberrant behaviour bf the chromosomes during mitosis or somatic mutations.

ENDOSPERM HAUSTORIA

All the three types of endospem described above may develop special structure called haustoria which elongate considerably and invade the tissue in the seed and placenta. Haustoria are believed to absorb energy sources and metabolise them for the developing endosperm. A few interesting examples of endosperm haustoria are given below.

Endosperm with Chalazal Haustorium

In Grevillea robusra, a membex of Proteacae the endosperm is of the free nuclear type. Tbe upper part of endospexm becomes cellular, whereas the lower part develops into a coenocytic, coiled wonn-like shuaure called the 'vemifonn appendage'

It serves as an aggressive haustorium, invades the chalaza1 tissue and

transports nutrients to the main endosperm.

Endosperm with Micropylar and Chalaza1 Haustoria

Some plants develop haustoria from both micropylar and chalazal ends of endospem. In Nemophila aggressive haustoria arise from micropylar and chalazal ends. The chalazal haustorium sometimes gives out a prominent lateral branch which grows towards the funiculus so as to come in direct contact with the starchy tissue of the placenta . In Melampynun lineare, the micropylar haustorium comprises a single cell with many tubular processes which enlarge considerably and invade the tissue of the integument and funiculus. The chalazal haustorium is short and confined to the nucellar tissue only ..

Endosperm with Lateral Haustoria

In Monochoria, in which the endospenn development is of helobial type, the haustorium is neither chalazal nor miaopylar but lateral. The chalazal chamber does not grow further and contains only a few nuclei but the micropylar chamber shows active nuclear divisions ant develops two lateral outgrowths, one on either side of the chalazal chamber . These grow downwards and function as active haustoria invading the tissue of chalazal. Later, the main body of the endospem enlarges considerably and fuses with the haustoria.

Morphological nature of Endosperm

The morphological nature of endosperm in angiosperms has been a subject of much discussion in evolution. The endosperm in gymnosperms is a gametophytic (haploid) tissue as it develops directly by the continued free nuclear divisions of the functional megaspore. In angiosperms, however, it develops from the primary endospm nucleus which is normally formed by the fusion of two polar nuclei and a male nucleus and

hence it is neither haploid nor diploid but generally triploid. Some workers have suggested that the endosperm in angiosperms is a gametophytic tissue just like those of gymnosperms, the only difference being that its development remains arrested till the entry of the pollen tube into the ovule. Other embryologists have considered it as a second embryo or a maimed embryo. The most agreeable view regarding the morphological nature of the endosperm in angiosperm is that it is an undifferentiated tissue which shows different degrees of polyploidy and becomes physiologically.
By suppressing the growth of the embryo in a seed, it has been possible to induce ttiploid shoot bud development in the endosperm. However, truly triploid plants have not been obtained so far.

Topic- 9

Parthenocarpy

Plants produce fruits and seeds following pollination and fertilization. But, there are other ways fruits and seeds are produced. Parthenocarpy is the formation or development of fruit without fertilization. Apomixis, on the other hand, is the formation of seeds without fertilization. Let us understand more about each of the phenomenon.

That some plants produce fruits without seeds is a fact observed and recorded by the ancients, according to Sturtevant in 1890" is the introductory statement reported in Gustafson's comprehensive work regarding the subject of parthenocarpy (Gustafson, 1942). The reasons for such an interest are soon after explained "because seedless fruits were thought to be better and also because many varieties are self-sterile, necessitating the planting of more than one variety in an orchard to insure a profitable crop" (Gustafson, 1942).

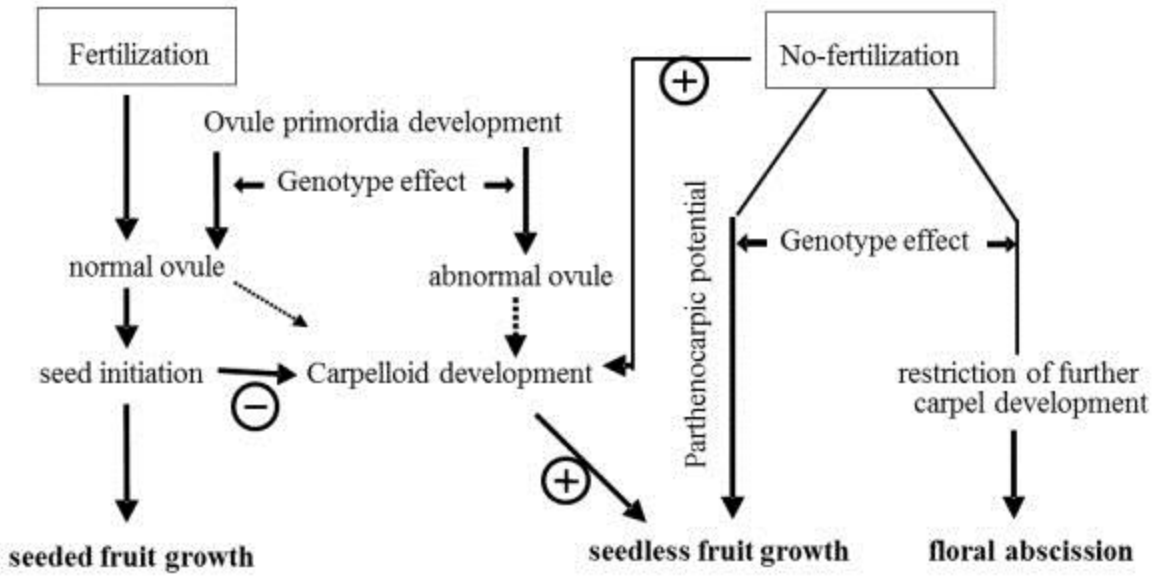
The production of seedless fruits (apireny or parthenocarpy sensu lato) has attracted since long time the farmers, because they are set independently of successful pollination. In addition, seedless fruits are favorable to processors, being their manipulation easier, and to consumers, being more pleasant to eat. Seedless fruits can occur when the ovary develops directly without fertilization (parthenocarpy sensu stricto) or when pollination and fertilization trigger ovary development, but the ovule/embryo aborts without producing mature seed (stenospermocarpy). The term parthenocarpy is hereby used in its broad sense to indicate both forms of apireny. Parthenocarpy is generally driven by genetic factors; nonetheless, seedlessness can be also induced with the application of various hormones to young inflorescences (Nitsch, 1952; Schwabe and Mills, 1981). Sources of genetic parthenocarpy are either obligate or facultative. In sexually propagated species, parthenocarpic genotypes should be facultative in order to be multiplied in conditions where the trait expressivity is lower. Differently, obligate parthenocarpy can be adopted in vegetatively propagated crops (Gorguet et al., 2005). From the adaptive point of view, the production of seedless fruits is an intriguing phenomenon, because empty fruits are costly to the mother plant and do not contribute to the production of offspring. When seed set fails, the abscission of the flower is the standard pathway to avoid the waste of resources in growing structures not fulfilling a biological purpose. The occurrence and permanence of parthenocarpy in plant populations is largely the effect of human selection that harnessed seedlessness as a commodity in fruit crops (Varoquaux et al., 2000). However, parthenocarpic genotypes are also found in wild species or in crops were the main product is not the fruit (non-

fruit crops), indicating the possibility of adaptive reasons underlying empty fruit formation in higher plants.

In parallel with parthenocarpy that involves carpel development independent of pollination, the term parthenogenesis is used to indicate the development of an embryo in absence of male contribution. Parthenogenesis is part of the process called apomixis, a modified mode of reproduction resulting in seed production by asexual means (agamospermy, Nogler, 1984). Seeds of apomictic origin replicate the exact genome of the mother plant as they result from the parthenogenetic development of unreduced (apomeiotic) egg cells. In gametophytic apomixis, the apomeiotic egg cell is differentiated within an unreduced female gametophyte developing when a somatic nucellar cell acquires the developmental program of a megaspore (apospory) or when the meiocyte bypasses meiosis and proceeds directly with the gametophytic development (diplospory). In all cases, apomixis opens the possibility for cloning genotypes by seed. By consequence, harnessing apomixis is an exciting perspective for plant breeders and efforts to decipher its genetic control have been strongly pursued in the last decades (Albertini et al., 2010).

The phenomenon of parthenocarpy and fruit production is popular among the horticulturalists. Since these varieties are very much preferred for the preparation of food items like jams, jelly, sauces, fruit drinks etc. The fleshy part of the fruit is also increased in accordance with the absence of the seed. **These are some advantages of parthenocarpy.**

As a result of parthenocarpy, parthenocarpic fruits are produced which are seedless so they are not a means for the reproduction. Applications of chemical are required for the production of fruits in the chemically induced parthenocarpy. Early application of auxins like phytohormones before anthesis damages the flowers. It results in the abortion of seed and fruit drop. **These are some disadvantages of parthenocarpy.**



Sequence for the development of Parthenocarpy

Topic-10

Polyembryony

The phenomenon of the development of more than one embryo in one ovule, seed or fertilized ovum is called polyembryony. It occurs in both animals as well as plants. Most striking cases of polyembryony are seen in certain animals {e.g. parasitic Hymenoptera), where up to 2,000 embryos may spring from one zygote.

In plants, this phenomenon was first reported by Antoni van Leeuwenhoek (1719) in orange seeds. In several gymnosperms, the polyembryony is so common that it might be regarded as an important character of this group.

Types of Polyembryony:

Polyembryony may be of following two types:

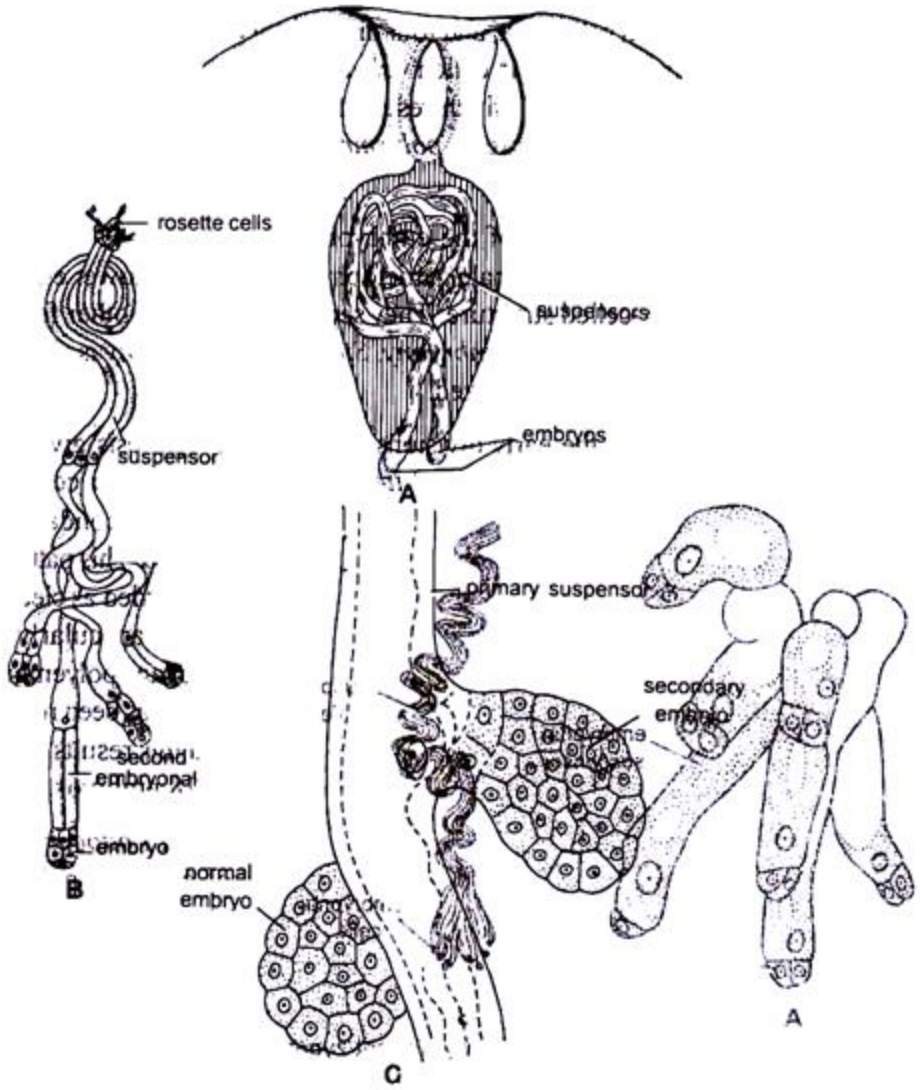
1. Induced Polyembryony : It includes cases of experimentally induced polyembryony.

2. Spontaneous Polyembryony: It includes all cases of naturally occurring polyembryony.

1. Cleavage Polyembryony: In this type a single fertilized egg gives rise to number of embryos.

2. Simple Polyembryony: In this type number of embryos develop as a result of the fertilization of several archegonia.

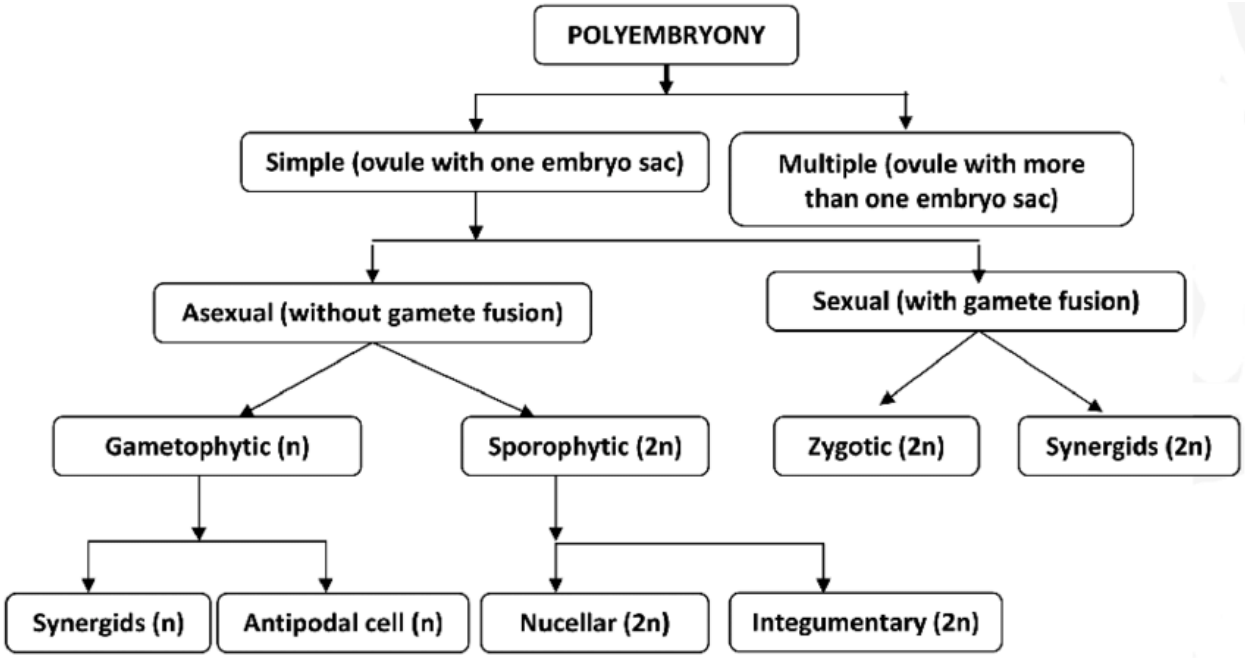
3. Rosette Polyembryony: In some gymnosperms (e.g. a few species of Pinus), additional embryos develop from the rosette cells, and this type of polyembryony has been termed as rosette polyembryony



Polyembryony in Angiosperm

Advantage of Polyembryony

Nucellar adventive polyembryony is of great significance in horticulture. The adventive embryos provide uniform seedlings of parental type. Nucellar seedling of Citrus provides better clones than cuttings. Cuttings form lateral roots and nucellar seedlings develop tap roots (better root system). Nucellar seedlings show restoration of vigour. Moreover, nucellar embryos are free from disease


POLYEMBRYONY
Simple (ovule with one embryo sac)
Multiple (ovule with more than one embryo sac)
Asexual (without gamete fusion)
Sexual (with gamete fusion)
Gametophytic (n)
Sporophytic (2n)
Zygotic (2n)
Synergids (2n)
Synergids (n)
Antipodal cell (n)
Nucellar (2n)
Integumentary (2n)

Topic-11

Experimental Embryology

A branch of embryology that studies the mechanisms controlling the individual development of animals and plants by means of experiments on living organisms is called as Experimental embryology. It uses such methods as marking, removal, transplantation, and isolation of body parts and organ

The first experiments on the developing embryo were followed by many others, and soon a new science was born – experimental embryology.

Experimental embryology, in contrast to comparative embryology or descriptive embryology, uses experiment as a method of investigation. However, the use of the experimental method in itself does not create a science or a branch of science. New branches of science are created by novel viewpoints and novel problems set before science.

It was the problem of what ontogenetic development actually is, what the driving forces behind it are, that necessitated the application of experiment after the methods of speculation and of pure observation were found to be impotent in solving the problem.The experimental embryology of plants can be discussed under following headings:

1.Evolution and Special Modifications for Adaptations: Morphological and anatomical adaptation in Hydrophytes, Xerophytes .

2: Stamen and Androecium: Development, Evolution and Dehiscence of Anthers –Development of the anther from the floral parts. Formation of Filament, connective and anther bearing pollen grains.

3: Male Gametophyte or Microsporogenesis : Development of Microspore and its germination to give rise to Male gametes.

4: Carpel and gynoecium: Structure and function,Evolution and Development Formation of ovary, style and stigma.. Formation of Megaspore..

5: Female gametophyte: Megasporogenesis : Development of embryo sac.

6: Pollination and Fertilization: Transfer of pollen grain to pistil and fertilization

7: Development of Seed and Fruit . Different types of seeds and development of different types of fruits.

8: Fruit and Seed dispersal : Maturation of different types of fruits and dispersal of seed

9: Applications of Plant Embryology. Application of Biotechnological principle such as:

a) Stem , root and leaf culture.

b) Anther culture

c) Pollen culture.

d) ovule culture

e) Haploid plant culture

f) Embryo culture.

10. Application Plant Genetic Engineering

Topic-12

Culture media for micropropagation

The culture media usually contain the following constituents:

1. Inorganic nutrients

2. Carbon and energy sources

3. Organic supplements

4. Growth regulators

5. Solidifying agents

6. pH of medium

Inorganic Nutrients:

The inorganic nutrients consist of macronutrients and micronutrients. A wide range of mineral salts (elements) supply the macro- and micronutrients. The inorganic salts in water undergo dissociation and ionization. Consequently, one type of ion may be contributed by more than one salt. For instance, in MS medium, K+ ions are contributed by KNO3 and KH2PO4 while NO3– ions come from KNO3 and NH4NO3.

Macronutrient elements:

The six elements namely nitrogen, phosphorus, potassium, calcium, magnesium and sulfur are the essential macronutrients for tissue culture. The ideal concentration of nitrogen and potassium is around 25 mmol I-1 while for calcium, phosphorus, sulfur and magnesium; it is in the range of 1-3 mmol I–. For the supply of nitrogen in the medium, nitrates and ammonium salts are together used.

Micronutrients:

Although their requirement is in minute quantities, micronutrients are essential for plant cells and tissues. These include iron, manganese, zinc, boron, copper and molybdenum. Among the microelements, iron requirement is very critical. Chelated forms of iron and copper are commonly used in culture media.

Carbon and Energy Sources:

Plant cells and tissues in the culture medium are heterotrophic and therefore, are dependent on the external carbon for energy. Among the energy sources, sucrose is the most preferred. During the course of sterilization (by autoclaving) of the medium, sucrose gets hydrolysed to glucose and fructose. Becomes the primary source of energy for the ex-plant.

Organic Supplements:

The organic supplements include vitamins, amino acids, organic acids, organic extracts, activated charcoal and antibiotics.

Vitamins:

Plant cells and tissues in culture (like the natural plants) are capable of synthesizing vitamins but in suboptimal quantities, inadequate to support growth. Therefore the medium should be supplemented with vitamins to achieve good growth of cells. The vitamins added to the media include thiamine, riboflavin, niacin, pyridoxine, folic acid, pantothenic acid, biotin, ascorbic acid, myo-inositol, Para amino benzoic acid and vitamin E.

Amino acids:

Although the cultured plant cells can synthesize amino acids to a certain extent, media supplemented with amino acids stimulate cell growth and help in establishment of cells lines. Further, organic nitrogen (in the form of amino acids such as L-glutamine, L-asparagine, L-arginine, L-cysteine) is more readily taken up than inorganic nitrogen by the plant cells.

Organic acids:

Addition of Krebs cycle intermediates such as citrate, malate, succinate or fumarate allow the growth of plant cells. Pyruvate also enhances the growth of cultured cells.

Organic extracts:

It has been a practice to supplement culture media with organic extracts such as yeast, casein hydrolysate, coconut milk, orange juice, tomato juice and potato extract. It is however, preferable to avoid the use of natural extracts due to high variations in the quality and quantity of growth promoting factors in them. In recent years, natural extracts have been replaced by specific organic compounds e.g., replacement of yeast extract by L-asparagine; replacement of fruit extracts by L-glutamine.

Activated charcoal:

Supplementation of the medium with activated charcoal stimulates the growth and differentiation of certain plant cells (carrot, tomato, orchids). Some toxic/inhibitory compounds (e.g. phenols) produced by cultured plants are removed (by adsorption) by activated charcoal, and this facilitates efficient cell growth in cultures. Addition of activated charcoal to certain cultures

(tobacco, soybean) is found to be inhibitory, probably due to adsorption of growth stimulants such as phytohormones.

Antibiotics:

It is sometimes necessary to add antibiotics to the medium to prevent the growth of microorganisms. For this purpose, low concentrations of streptomycin or kanamycin are used. As far as possible, addition of antibiotics to the medium is avoided as they have an inhibitory influence on the cell growth.

Growth Regulators:

Plant hormones or phytohormones are a group of natural organic compounds that promote growth, development and differentiation of plants. Four broad classes of growth regulators or hormones are used for culture of plant cells-auxins, cytokinins, gibberellins and abscisic acid. They promote growth, differentiation and organogenesis of plant tissues in cultures.

Auxins:

Auxins induce cell division, cell elongation, and formation of callus in cultures. At a low concentration, auxins promote root formation while at a high concentration callus formation occurs. Among the auxins, 2, 4-dichlorophenoxy acetic acid is most effective and is widely used in culture media.

Cytokinins:

Chemically, cytokinins are derivatives of a purine namely adenine. These adenine derivatives are involved in cell division, shoot differentiation and somatic embryo formation. Cytokinins promote RNA synthesis and thus stimulate protein and enzyme activities in tissues. Among the cytokinins, kinetin and benzyl-amino purine are frequently used in culture media.

Ratio of auxins and cytokinins:

The relative concentrations of the growth factors namely auxins and cytokinins are crucial for the morphogenesis of culture systems. When the ratio of auxins to cytokinins is high, embryogenesis, callus initiation and root initiation occur.

On the other hand, for axillary and shoot proliferation, the ratio of auxins to cytokinins is low. For all practical purposes, it is considered that the formation and maintenance of callus cultures require both auxin and cytokinin, while auxin is needed for root culture and cytokinin for shoot culture. The actual concentrations of the growth regulators in culture media are variable depending on the type of tissue explant and the plant species.

Gibberellins:

About 20 different gibberellins have been identified as growth regulators. Of these, gibberellin A3 (GA3) is the most commonly used for tissue culture. GA3 promotes growth of cultured cells, enhances callus growth and induces dwarf plantlets to elongate. Gibberellins are capable of promoting or inhibiting tissue cultures, depending on the plant species. They usually inhibit adventitious root and shoot formation.

Abscisic acid (ABA):

The callus growth of cultures may be stimulated or inhibited by ABA. This largely depends on the nature of the plant species. Abscisic acid is an important growth regulation for induction of embryogenesis.

Solidifying Agents:

For the preparation of semisolid or solid tissue culture media, solidifying or gelling agents are required. In fact, solidifying agents extend support to tissues growing in the static conditions.

Agar:

Agar, a polysaccharide obtained from seaweeds, is most commonly used as a gelling agent for the following reasons.

1. It does not react with media constituents.

2. It is not digested by plant enzymes and is stable at culture temperature.

Agar at a concentration of 0.5 to 1% in the medium can form a gel.

pH of medium:

The optimal pH for most tissue cultures is in the range of 5.0-6.0. The pH generally falls by 0.3-0.5 units after autoclaving. Before sterilization, pH can be adjusted to the required optimal level while preparing the medium. It is usually not necessary to use buffers for the pH maintenance of culture media.

At a pH higher than 7.0 and lower than 4.5, the plant cells stop growing in cultures. If the pH falls during the plant tissue culture, then fresh medium should be prepared. In general, pH above 6.0 gives the medium hard appearance, while pH below 5.0 does not allow gelling of the medium.

Procedure for preparation of Culture media.

Ingredients are mixed in suitable quantity dispensed in glassware and sterilized. Vitamin solution, growth regulators are mixed prior to plating.

Students are further advised to prepare notes on Development of Male gametophyte and Female gametophyte in Angiosperm