TOPIC :- LANDSCAPE GARDENING

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Department of Botany

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M.Sc, SET

Definition:-

- A garden may be defined as a place for growing plants, exhibits various forms of plant life, which are consciously directed for ornamental or practical use.
- Now a days we often use the term landscape gardening ,landscape architecture, landscape design etc.
- Arrangement of trees, shrubs, climbers and various other plants together with the building, walks, drives, artificial and natural features for the use of humanity is termed as "**Landscape gardening".**
- **"Landscape architecture"** is the art of arranging land and landscape for human use, convenience and enjoyment.

Landscape gardening

Arrangement of trees, shrubs, climbers and various other plants together with the building, walks, drives, artificial and natural features for the use of humanity.

Scope of landscape gardening

- Towns with open spaces around the building and their terraces, industries with their specific problems of controlling pollution, amusement parks, hotels, roadways, riverbanks etc. are some area require the services of a landscape gardening.
- The landscape gardening become a profession.
- It is the art of arranging land and landscape for human use, convenience and enjoyment.
- It is concerned with the individual home and the whole community.
- It provide scope for diversification in agriculture sector

Scope of landscape gardening

- Earning income through sales of landscape accessories, resources, garden tools, bio inputs.
- Generates extra income by starting nurseries, tissue culture units, by opening florists shop, plant rental services, by landscaping.
- Scope to extract essential oils from the flowers and also make dry flowers which can be a source of income and employment
- India has tremendous potential of growing ornamental crops because it is endowed with diverse agro-climatic conditions which help to grow various kinds of flowers almost throughout the year in one part of the country or the other. There is scope for expanding area under flowers.

Scope of landscape gardening

- It creates employment because floriculture is labour intensive, it provides more farm employment to rural and urban population by way of growing and selling floricultural products.

Objectives of landscape gardening

- The objective of the landscape are to integrate functionally and aesthetically, the people, building and site.
- Plants and space are the main tool to achieve these objectives.
- Landscape architecture is closely linked to arts, architecture, planning, ecology, sociology and horticulture. As a result, landscape architects are increasingly being involved in interdisciplinary projects.
- To design the site with more natural aspects.
- To make the site more comfortable.
- Restore environmental aspects via designing.
- Maintain own characteristics of the site.
- To keep natural elements and decorate them.

OUTDOOR GARDEN

1) Lawns:-

- Lawn is the most important elements in the garden design, attractive at all the times providing a pleasant surroundimng for house, trees, shrubs and flowers.
- A **lawn** is an area of soil-covered land planted with grasses which are maintained at a short height with a lawnmower (or sometimes grazing animals) and used for aesthetic and recreational purposes.
- Lawns are usually composed only of grass species maintained in a green color by watering and are regularly mowed to ensure an acceptable length.
- Lawns are used around houses, apartments, commercial buildings and offices.
- Many city parks also have large lawn areas.
- In recreational contexts, the specialised names **turf**, **pitch**, **field** or **green** may be used, depending on the sport and the continent.

Preparation of lawn:-

Site and soil-

- After choosing the site, the next important factor for consideration is the size and shape of the lawn.
- The preparation of site includes digging, leveling and enriching the soil with organic manures or by amending with fertile soil.
- The ideal soil pH should be 5.0 to 5.6.
- The size of the lawn it depend very much on the availability of space, whereas the shape should be such that it creates an attractive appearance.

Lavelling-

- The site should be thoroughly levelled with spade, pebbles and weeds are hand picked.
- The soil is rolled with a roller.
- Weeds especially nut grass should not be allowed to grow and should be removed with roots for at least 2 to 3 times.

Methods-

1.Seedling

- About 30 kg of seed is required for planting one hectare.
- The soil should be reduced to fine tilth and given a light rolling.
- The site should be divided into suitable small squares or rectangles, the seeds are mixed with double the quantity of finely sieved soil and should be rolled again and watered liberally .
- The seeds take four to five weeks for germination.
- For the first few times, the grasses are cut with a scythe. Lawn mower may be used for easy maintenance and for its spreading.

Recovery Fast Growing Grass Seeds

DEFRA certified quality seeds

KINGDOM

GARDEN & LANDSCAPING

2.Turfing-

- The turfs are nothing but pieces of earth with compact grasses on them.
- These turfs should be cut uniformly in squares from a place where the grass is short, compact and free from weeds.
- This is the most expensive way of lawn making.

3.Turf plastering-

- The doob grass can be procured in large quantities free from weeds and chopped properly into small bits of 5-7 cm long.
- Two baskets of chopped grass pieces should be mixed well with one basket each of garden soil and fresh cow dung and a shovel full of wood ash with required quantity of water to form a thick pasty substance.
- This mixture is then spread uniformly on the surface.
- The next day, ground should be rolled and the grass should be allowed to spread.

4.Dibbling roots-

- This is the cheapest but time consuming method.
- Small pieces of grass roots should be dibbled 10 – 15 cm apart in a leveled ground when it is wet after rain.
- The roots spread and grow underground in the course of six months making a fairly compact lawn by frequent mowing, rolling and watering.

Lawn types-

1.Warm season grasses

- Warm season grasses exhibit active growth between the months of April and October and are both heat and drought tolerant.
- e.g-Bahia Grass, Bermuda Grass

2.Cool Season Grasses

- Cool season grasses are the ideal choice in areas with extreme seasonal fluctuations where summers are hot and dry and winter temperatures fall below freezing.
- e.g- Fine Fescue, Kentucky Bluegrass

Rock Garden

- Rock garden is the interesting feature in the garden.
- In laying out a garden to bring nature in a home and like other features introduce rocks in the garden and feel happy to see plants growing well on rocks.
- In nature rocks serve as a pots.
- To select rocks from material which is porous and soil in rock garden must be well drained.
- The aim should be imitate a piece of nature.
- If associayted with walls, green houses por other artificial surroundings the illusion falls to pieces.
- The rock garden may shape of mountain or the stonmy slope of a hill, a rock creast or a peak.
- Careful selection of plants and bulbs suitable for growing in rocks which give long flowering season and beautiful colour effect are desirable

Rock Garden

Water garden

- In landscape gardening use of water in their setting plays a prominent role for many reasons.
- Garden designers have always produced the most satisfactory results, when they have been able to bring water in some way inmto their compositions.
- It is beautiful and attractive at all times and providing a pleasant everchanging surface varying with the change of season and weather.
- The beauty of water-side planting is enhanced by charming reflections.
- Lovely hybrids of water-lilies and waterloving plants are gaining much importance in modern gardening.
- If any site of a garden, natural stream, pond or lake exists, water garden is at once assured by planting suitrab le plants. If no such natural facilities exist, an artificial pool must be formed in a sunny spot.The music of flowing water, the beautiful forms which can be made to rise and fall anmd the association of architectural designs account for the deserved popularity of the fountain.
- The success of fountain depends upon the water at sufficient pressure and skilfull plumbing.
- If surrounded by alarge basin or pond, upright jets may be adopted for smaller fountains, some simple form of bubbles or hets thrown out from the side of a central arrangement would be found most satisfactory.

Water garden

Terrace Garden

- A terrace garden is a garden which is established on a terrace, roof, or patio, usually in a house where there is limited gardening space.
- These types of gardens are especially popular in urban areas, and they are sometimes used in restaurants and other establishments as well.
- Terrace gardens in the sense of patio or rooftop gardens can be ornamental or functional, and they are usually designed with container plants to make the terrace easier to manage.
- Gardeners can also build raised beds on their terraces for gardening.
- Sun exposure and access to water are two important things to consider when establishing the garden, as is the goal of the garden.
- Some people like to grow fresh herbs and vegetables on their terraces, while others want to create a green retreat. Occasionally, gardeners create a mixture of the two.
- Plants can be trellised around a terrace for privacy or shade, or used to create a pleasant recreation area for residents and guests of the home.
- It pays to plan ahead when developing this type of garden, because of space considerations.

Terrace Garden

Green house

- A **greenhouse** is a structure with walls and roof made chiefly of transparent material, such as glass, in which plants requiring regulated climatic conditions are grown.
- These structures range in size from small sheds to industrial-sized buildings.
- A miniature greenhouse is known as a cold frame.
- The interior of a greenhouse exposed to sunlight becomes significantly warmer than the external temperature, protecting its contents in cold weather.
- Many commercial glass greenhouses or hothouses are high tech production facilities for vegetables, flowers or fruits.
- The glass greenhouses are filled with equipment including screening installations, heating, cooling, lighting, and may be controlled by a computer to optimize conditions for plant growth.
- Different techniques are then used to evaluate optimality degrees and comfort ratio of greenhouses, such as air temperature, relative humidity and vapour-pressure deficit, in order to reduce production risk prior to cultivation of a specific crop.

Green house and polyhouse

INDOOR GARDEN

- Indoor plants:-

Indoor gardening is a technique used to grow plants indoors. Hydroponic gardening uses no soil at all. Instead, it relies on growing plants in water and the use of additives to provide the nutrients that would come from soil if the plants were grown outdoors.

VIVEKANAND COLLEGE KOLHAPUR (AUTONOMOUS)

Class: B.Sc-I

Topic Name: Study of *cycas*

Name of teacher- Miss. P. B. Shelar

M.sc., MH-SET

Study of *Cycas*

Classification-

Kingdom- Plantae (Cell wall is made up of Cellulose)

Sub kingdom- Phanerogams (Flowering plants)

Division- Gymnosperms (Seeds nacked)

Class- Cycadopsida (small tree, unbranched stem covered by leaf bases, pinnately compound leaves.)

Order- Cycadales (Young leaves circinately coiled, ovule is orthrotropous)

Family-Cycadaceae (Corolloid roots present)

Genus- *Cycas*

Species- *revoluta*

General Characters-

- Plant is unbranched with aerial stem and pinnately compound leaves.
- Roots are of two types i.e. Primary tap roots and secondary coralloid roots (growing near soil surface)
- The stem is covered by persistant leaf bases and hard scaly leaves.
- Leaves are of two types i.e Large pinnately and green foliage leaves and brown, hard small scaly leaves.
- Each pinnae in a foliage leaves is tough leathery entire with distinct midrib and without side veins.

Reproductive Characters

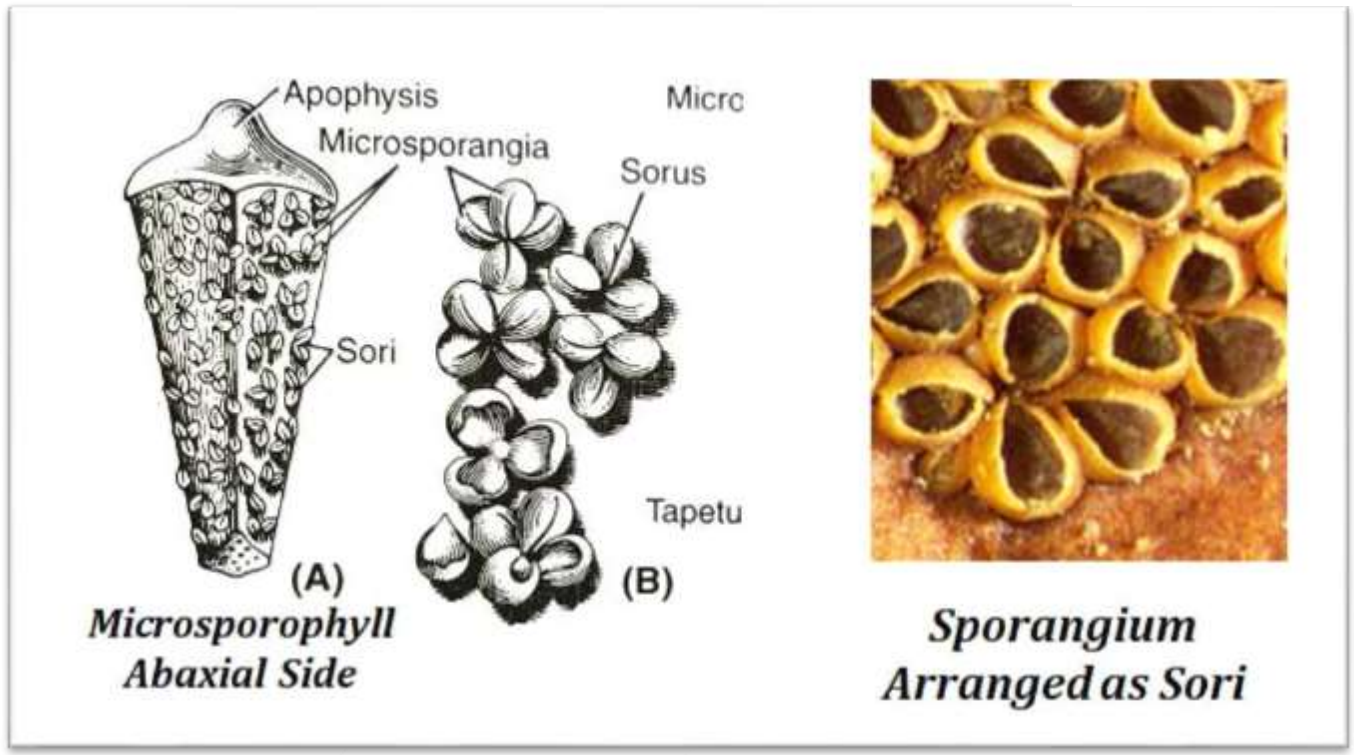
- *Cycas* is a dioecious produces male and female reproductive structure on separate plant.

Male cone-

- It is terminal in position with short stalk, oval in shape.
- It consist of central axis around which many microsporophylls are arranged spirally.
- Each microsporophyll shows terminal sterile region called apophysis.
- Microsporangium are present of lower surface in the form of growth called sori. Each microsporangium enclosed microspores or pollens.

Microsporophyll

Cycas: Pollen Grains

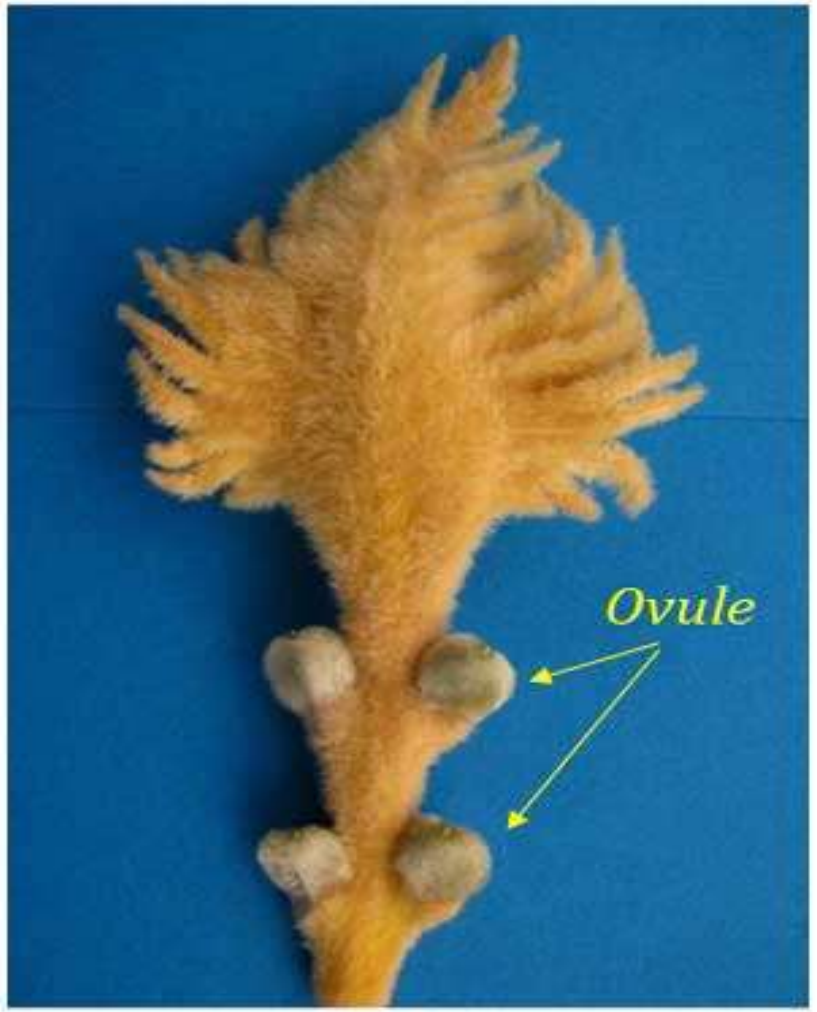


Microsporophyll
Abaxial Side

Sporangium
Arranged as Sori

Female cone-

- In case of *Cycas* megasporophyll grows separately without forming a cone.
- They are present in a group in terminal position.
- Each megasporophyll is leaf like covered with brown hairs showing lower petiole and middle ovule bearing region and upper sterile region.
- The ovules are produced in two rows on the either side of megasporophyll.



Cycas Megasporophyll with Ovules

VIVEKANAND COLLEGE KOLHAPUR (AUTONOMOUS)

Class: B.Sc-III

Topic Name: Plant Tissue Culture

Name of teacher- Miss. P. B. Shelar

Botany Paper- VIII
Section-I
Unit IV: Plant Tissue Culture

4a.: Principle and Totipotency

4b.: Components of tissue culture media, Sterilization techniques

4c.: Techniques in Tissue culture (Callus culture and Cell suspension)

4d.: Organogenesis and Embryogenesis

4e.: Anther culture

4f.: Applications of Plant Tissue Culture

Terminology used in PTC

- **Totipotency:**

Latin word- 'Totipotentia' means ability for all things.

"A living, nucleated plant cell which is capable of dividing, contain a complete information for the development of new plant in its genome. Under ideal conditions such cell can regenerate and develop into a complete new plant. This property of the cell is known as totipotency."

- **Callus:** It is an unorganized mass of thin walled cells or a tissue in which the cells are loosely arranged and undifferentiated.
- **Clone:** A group of plants multiplied vegetatively from a single plant is a clone.
- **Cybrid:** A hybrid which is produced by the fusion of isolated protoplast in culture media belonging to different genera or species is called as cybrid.
- **Cultivar:** The plant which is selected for the tissue culture is called as cultivar.
- **Explant:** The protoplast, cell, tissue or organ of the plant used for tissue culture is called explant.
- **Organogenesis:** Formation of organs such as root, shoot from the callus in PTC is called organogenesis.

- **Sub-culture:** Transfer of a fragment of the parent culture to a new medium is called as sub-culture.

- **Inoculation:** The sterilized material (explant) carefully transferred to the test tubes or suitable container and put on the culture medium with the help of sterilized forceps. This process of transformation of explant on nutrient medium under asepetic conditions is known as inoculation.

- **Incubation:** The process of keeping the inoculum under ideal conditions (proper temperature, light etc.) or Conditions required for the development of explant into callus the time period is known as incubation.

- **Plantlet:** The plant obtained through the tissue culture technique is called plantlet.

- **Hardening:** The introduction of tissue-cultured plantlets into the soil by gradual acclimatization technique called as hardening.

Tissue Culture / *In-vitro* culture:

"The technique of separation of cells or tissues or organs of a plant and growing them aseptically in suitable glass container on a sterile nutrient medium under controlled conditions of temperature and light is called as tissue culture."

HISTORY

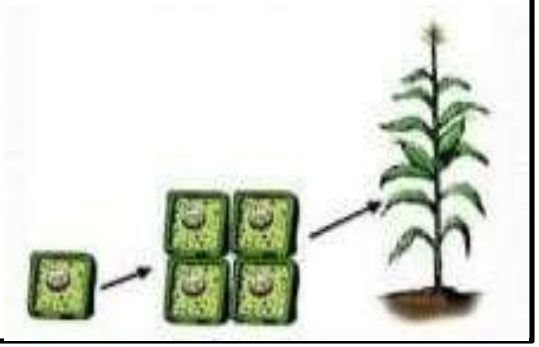
1902	The idea of the totipotency of plant cell was given by Haberlandt
1937	White first time established successful root culture of tomato
1941	Vanoverbeek used coconut milk for growth and development of young Datura embryos
1957	Skoog and Miller demonstrated the role of auxin and cytokinin on root and shoot formation in tobacco – tissue
1962	Murashige and Skoog introduced the medium for tobacco culture
1987	Isolation of Bt. gene form bacterium *Bacillus thuringiensis*

Principle of PTC

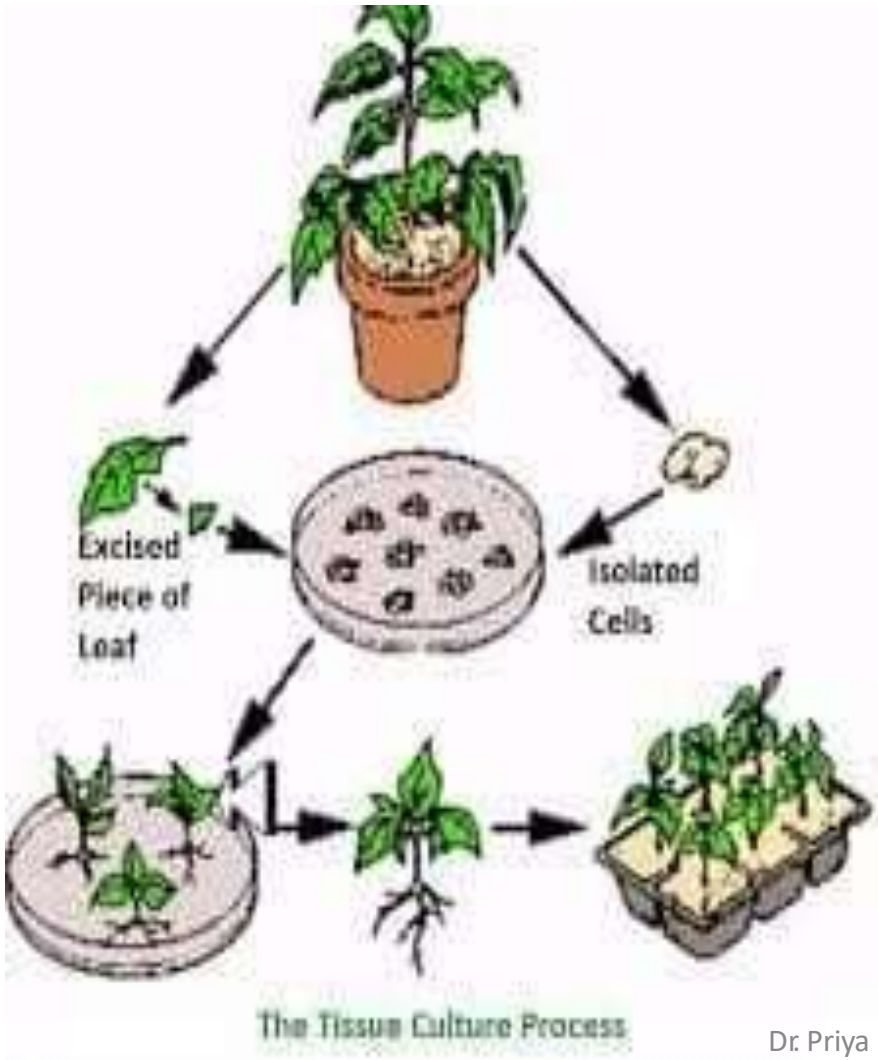
- PTC depends on-

1) **Totipotency:** It is ability of plant cells to regenerate into a whole plant.

2) **Plasticity:** It is the ability of plants to alter their metabolism, growth and development to best suit their environment.



Steps involved in PTC



- **Selection of explant:**

The explant which is selected it may either haploid or diploid explant.

- **Sterilization:**

All thermomaterials e.g. vessels, instruments, medium, plant material etc. used in culture work must be free from microbes.

Steps in PTC

Selection of explant

↓

Isolation of explant

↓

Sterilization of explant

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Inoculation of explant

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Incubation

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Initiation of callus

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Sub-culturing

↓

Regeneration

↓

Hardening

↓

Transfer of plantlets to Green house or open field

Factors Affecting Tissue Culture Efficiency

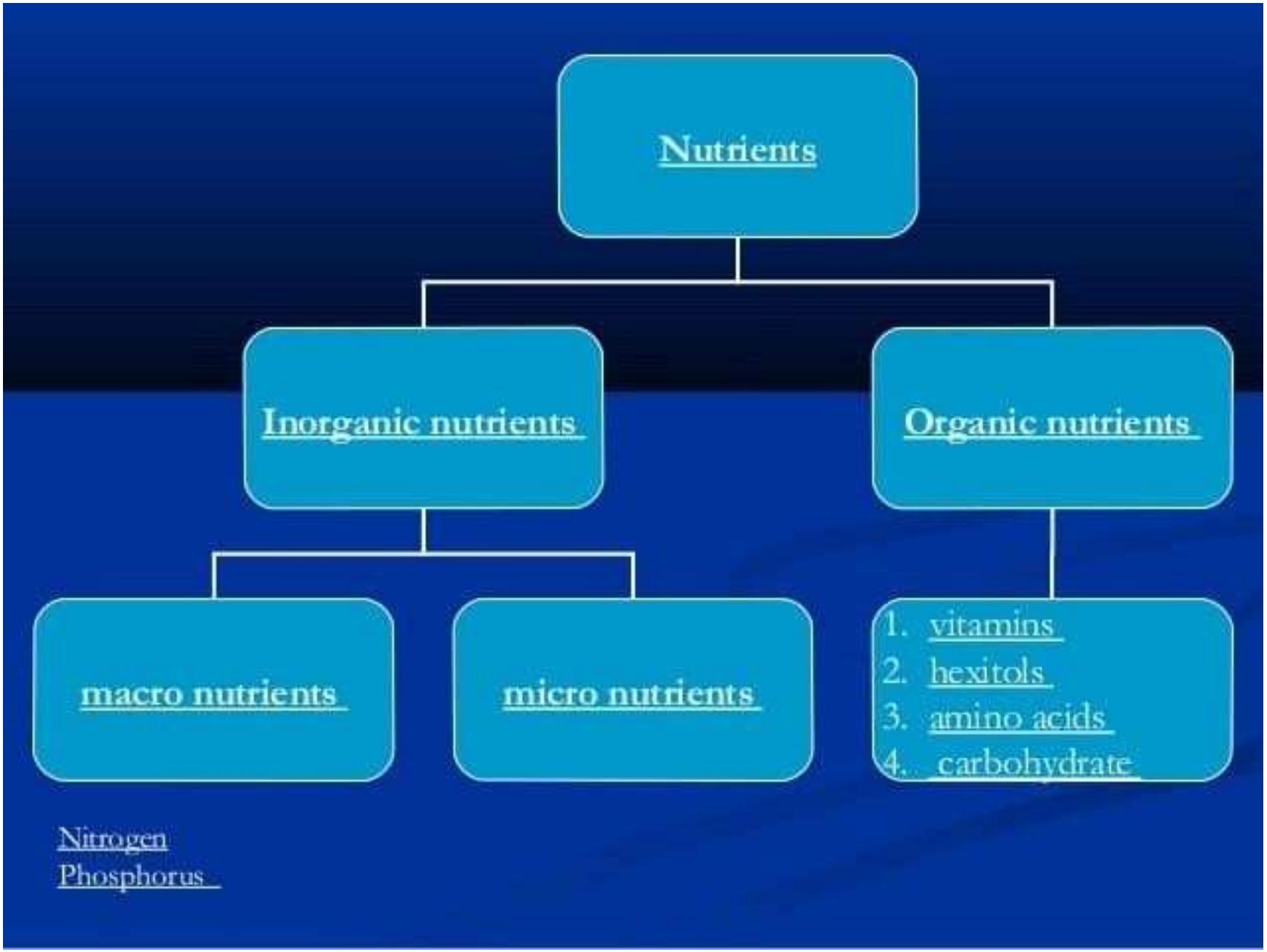
Plant regeneration from tissue culture varies with the following parameters:

- plant species,
- genotype within the species,
- source of the cultured tissue,
- age and health of the donor plant,
- nutrient medium, other factor

Plant Tissue Culture medium

• Media used in PTC contain nutritional components.
• Nutritional components are essential for growth and development of cultured tissue.
•The success of the tissue culture depends on the type of culture media used.
• Each PTC medium must contain the following essential components to support *in vitro* plant growth.
•PTC medium consists of :

i) Macro inorganic nutrients
ii) Micro inorganic nutrients
iii) Iron (as a chelating agent)
iv) Vitamins
v) Carbon source
vi) Organic nitrogen
vii) Plant growth regulators
viii) Agar (as a gelling substance)


Nutrients
Inorganic nutrients
Organic nutrients
macro nutrients
micro nutrients
1. vitamins
2. hexitols
3. amino acids
4. carbohydrate
Nitrogen
Phosphorus

i) Macro nutrients:

- Required in higher concentration.
- It is present in medium in milli molar (mM) quantities
- Macro nutrients provide both anions and cations for the plant cells.
- e.g. Nitrogen (as NO_3 and NH_4), Phosphorus (PO_4), Potassium (K), Sulphur (SO_4), Magnessium (Mg) and Calcium (Ca).
- These have structural and functional role in protein synthesis, cell wall synthesis, enzyme co-factors and membrane integrity.

❖ Nitrogen:

•In organic form used as amino acids, different organic acids and caesin hydrolysate.

• In inorganic form used as Nitrate or ammonia.

• Nitrogen is a major component of all PTC media

• Nitrogen helps to synthesis complex organic molecule.

❖ Potassium:

•K ion present in high concentration in the cytoplasm (100-200 mM) and in chloroplast (20-200mM) .

•It is essential for maintaining the ion balancing, activation of many enzymes, maintaining osmotic pressure and osmotic regulation of cells.

❖ Calcium:

•Calcium functions with different enzymes as co-factor and bound to the cell wall and cell membrane.

• It gives strength to the cell wall.

• It helps in regulation of the cell membrane structure.

• Deficiency causes disintegration of the membrane and shoot tip necrosis.

• Important in cell and root multiplication.

• Supplied as calcium chloride and calcium nitrate.

❖ Phosphorus:

- Very important for energy metabolism.
- Essential element for DNA and RNA.
- Deficiency may cause delayed growth.
- Supplied as sodium hydrogen phosphate or potassium hydrogen phosphate.

❖ Magnessium:

- Essential for enzymatic reactions, energy metabolism (ATP Synthesis).
- Supplied as magnessium sulphate.

❖ Sulphur:

- Important substance.
- Deficiency inhibits protein synthesis and decreases chlorophyll in leaves.
- Supplied as magnessium sulphate and Potassium sulphate.

ii) Micro nutrients:

- Required in less concentration.
- It is present in medium in micro molar (µM) quantities
- e.g. Boron (B), Manganese (Mn), Zinc (Zn), Molybdenum (Mo), Copper (Cu), Cobalt (Co) etc.
- Used in less amount less than 30 ppm (mg/l).

❖ Zinc:

• Zn plays an active role in protein synthesis and in the synthesis of tryptophan.

• Supplied as zinc sulphate.

❖ Manganese:

- Plays an important role in the Hill reaction of Photosynthesis.
- Required in many enzymatic activities.
- Supplied as Manganese sulphate.

❖ Boron:

- Involves in different enzymatic activities.
- Supplied as Boric acid.

❖ Copper:

- Intermediate of the electron transport chain between photo system I and II.
- Deficiency leads to decrease in photosynthesis.
- Supplied as Copper sulphate.

❖ Molybdenum:

- Essential for conversation of Nitrate to Ammonium.
- Supplied as Sodium molybdate.

❖Iron:

- Important enzyme co-factor. Supplied in µM quantities.
- It is supplemented with chelators and complex compounds due to its solubility problem.
- Iron deficiency have severe effects on the growth and development plant cells.
- Supplied as $Na_2FeEDTA$.

Other Nutrients

1) Vitamins:

- Plant synthesis required vitamins.
- Essential for many biochemical reactions.
- Cultured cells are capable to produce vitamins at some level.
- They require an exogenous supply of different vitamins for optimum growth.
- Most usable vitamins are Thiamine, Pyridoxine, Nicotinic acid, Vitamin B Complex etc.

2) Hexitols:

- Most tissue culture media have this compound.
- Essential for seed germination, sugar transport, carbohydrate metabolism, membrane structure and cell wall formation.
- Mannitol and sorbitol are hexitols.

3) Amino acids:

- Glycine is the most common amino acid used in different culture media.
- It is not essential but Nitrogen containing amino acid enhance growth and plant regeneration.

4) Carbohydrate:

•Cells and tissue requires exogeneous supply of carbohydrates to replace the carbon which the plant normally fixes from the atmosphere by photosynthesis.

• Supplied by adding sucrose.

•Concentration is 20-30 gm/l.

5) Gelling agent:

• Agar-agar is used as a gelling agent.

• Agar is a natural product of seaweeds.

• Since 1658, agar-agar is obtain from red algae (*Gelidium* & *Gracillaria*).

•With water it melts at 100ºC and solidify at 45ºC.

6) Plant Growth Regulators:

- A plant hormone can be defined as a small organic molecule that elicits a physiological response at very low concentration.
- PGR's plays an important role in the phenotype.
- Act as messenger between environment and the genome.

- **Auxins:**
- Essential for cell division, cell elongation, cell differentiation, organogenesis and callus formation.
- Natural forms are IAA, IBA, PAA etc.
- Synthetic forms are NAA, 2,4-D.

• **Cytokinins:**

•Cytokinins promote cell division, shoot proliferation and influence the cell cycle.

• Promotes embryogenesis and inhibit root formation.

• Synthetic form is 2-ip, which is most active cytokinin.

• Natural forms are BAP and kinetin.

• **Gibberellins:**

• It promotes stem elongation, bulb corm formation and embryo maturation but can inhibit callus growth and root induction.

•GA3 is most common gibberellins.

• **Abscisic acid:**

• It inhibits shoot growth and germination of embryo.

•It is thermostable but light sensitive.

❖ pH of tissue culture media:

•pH is adjusted between 5 .5 to 5.8 before gelling and sterilization with the help of dilute NaOH, KOH or HCL.

•pH below 5 will not gel properly.

•pH above 6 may be too hard.

Sterilization Techniques

Sterilization is done for killing or removal of all micro-organisms, including bacterial spores. In PTC, sterilization is achieved by one of the following:

i) Dry heat treatment

ii) Flame sterilization

iii) Autoclaving

iv) Filter sterilization

v) Wiping with 70% ethanol

vi) Surface sterilization

i) Dry heat treatment:

Dry heat sterilization is done in hot air oven. Generally, used for glass equipments used in PTC. Dry heating generally achieved in 15 minutes at a temperature 160°C.

ii) Flame sterilization:

Metallic instruments *viz.* forceps, scalpel, needle, spatula and scissors are dipped in 70% ethanol followed by flaming with a burner (Bunsen burner or spirit lamp) and cooling.

(**Note:** Ethanol is volatile and inflammable. So this procedure involves the risk of fire during sterilizing the equipments.)

Figure: Commonly used instruments in plant tissue culture

iii) Autoclaving:

In autoclaving, pressurized steam is used to heat the material to be sterilized. Plant tissue culture media are generally sterilized by autoclaving at a temperature 121°C and 15 lbs pressure for 15 min.

Figure. Autoclave unit used for wet sterilization of nutrient media

iv) Filter sterilization:

Some growth regulators (GA3, urea, certain vitamins and enzymes) are heat labile. Such compounds are filter sterilized by passing their solution through a membrane filter of 0.45 micron or lower pore size.

Laminar air flow cabinets are used to create an aseptic working space by blowing filter-sterilized air through HEPA (High Efficiency Particulate Air) filter. This filter can remove 99.97% of dust, pollen, mold, bacteria and any airborne particles with a size of 0.3 µm.

iv) Wiping with 70% ethanol :

70% ethanol is effective to kill microbes, bacteria and other micro-organisms on the surfaces. Worker's hands and laminar flow cabinet occasionally sterilized by wiping with 70% ethanol.

v) Surface sterilization:

Surface sterilization is used for explant sterilization. For this, sodium hypochloride, hydrogen peroxide, mercuric chloride etc. are used in low concentration. Dipping the explant in 0.1% mercuric chloride for 5 min. followed by three times wash in distilled water is commonly used method.

Explant sterilization

Explant sterilization in Laminar Air Flow

The sterilizing agent used for surface disinfection are-

- Calcium Hypochloride (9-10%)
- Sodium Hypochloride (2%)
- Mercuric chloride (0.1-1%)

} Commonly used

- Silver nitrate (1%)
- Bromine water (1-2%)
- Hydrogen Peroxide (10-12%)

Techniques in tissue culture-

1. Callus culture-

- Callus is the initiation and proliferation of undifferentiated parenchymatous cells from an explant on a chemically defined medium in vitro.

- Almost any tissue can be used as an explant. However, when an explant comes from relatively young region, it will have a greater potential for cell division as well as regeneration.

- The explant should be small enough in order that it will be of fairly homogenous cells, but at the same time should be capable of sustained cell division.

Callus

- The medium should have a mixture of salts, harmones, suitable pH and agar for gelling.
- Callus cultures are incubated in dark.
- The explants generally used are shoots, buds, roots and embryos.
- These should be surface sterilized before inoculation.

Principle-

When an explant is inoculated on a nutrient medium supplemented with growth regulators and incubated, undifferentiated masses of tissue (callus) develop.

Requirements-

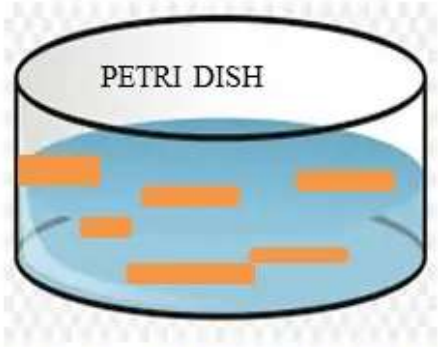
- Nutrient medium- Murashige and Skoog's(1962) medium supplemented with growth regulators.
- Explant-seeds of *Phaseolus.*
- Ethanol
- Sodium hypochlorite solution
- Sterile distilled water
- Sterile petriplates with moist filter paper
- 45% acetic acid
- Acetocarmine
- Slides and coverslips
- Inoculating needles
- Scalpel
- Long forceps
- Dark culture room at 25°C
- Laminar clean air flow hood.

WASH CARROT ROOT TIP

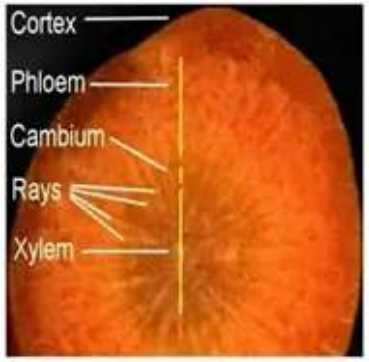
BREAK IT IN TO 3-4 PIECES

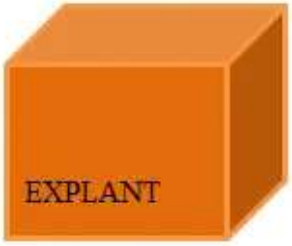
CUT THE CORE AND RINSE IT IN TO WATER

BALANCING THE PH BY ADDING
SOD.HYDROCHLORITE SOLN

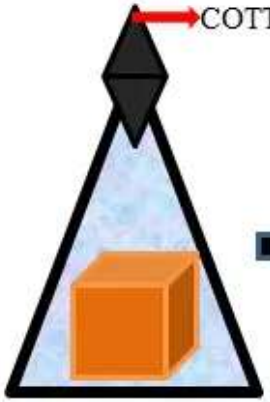


PLACE DISKS IN TO CALLUS INITIATION MEDIUM





TRANSFER OF EXPLANT USING
FORCEPS ONTO THE SURFACE OF
THE AGARIFIED NUTRIENT MEDIUM



CULTURE MEDIUM INCUBATED
OF 25^{0}C FOR 2-4 DAYS

CALLUS IS FORMED

Procedure-

- Sterilize healthy *Phaseolus* seeds with unbroken testa, by first washing in water and dipping them in 95% ethanol for 30 seconds.

- Transfer them to HgCl2 or sodium hypochlorite solution and keep for 5-10 min shaking occasionally.

- Rinse thoroughly with sterile distilled water, 5-6 times.

- Place them in sterile Petri plates with moist filter paper and incubate at room temperature in dark for 2-5 days.

- Cut hypocotyl, shoot tips or leaf segments measuring 5.0-7.0 mm using a sterile scalpel and use as explants to inoculate on nutrient medium.

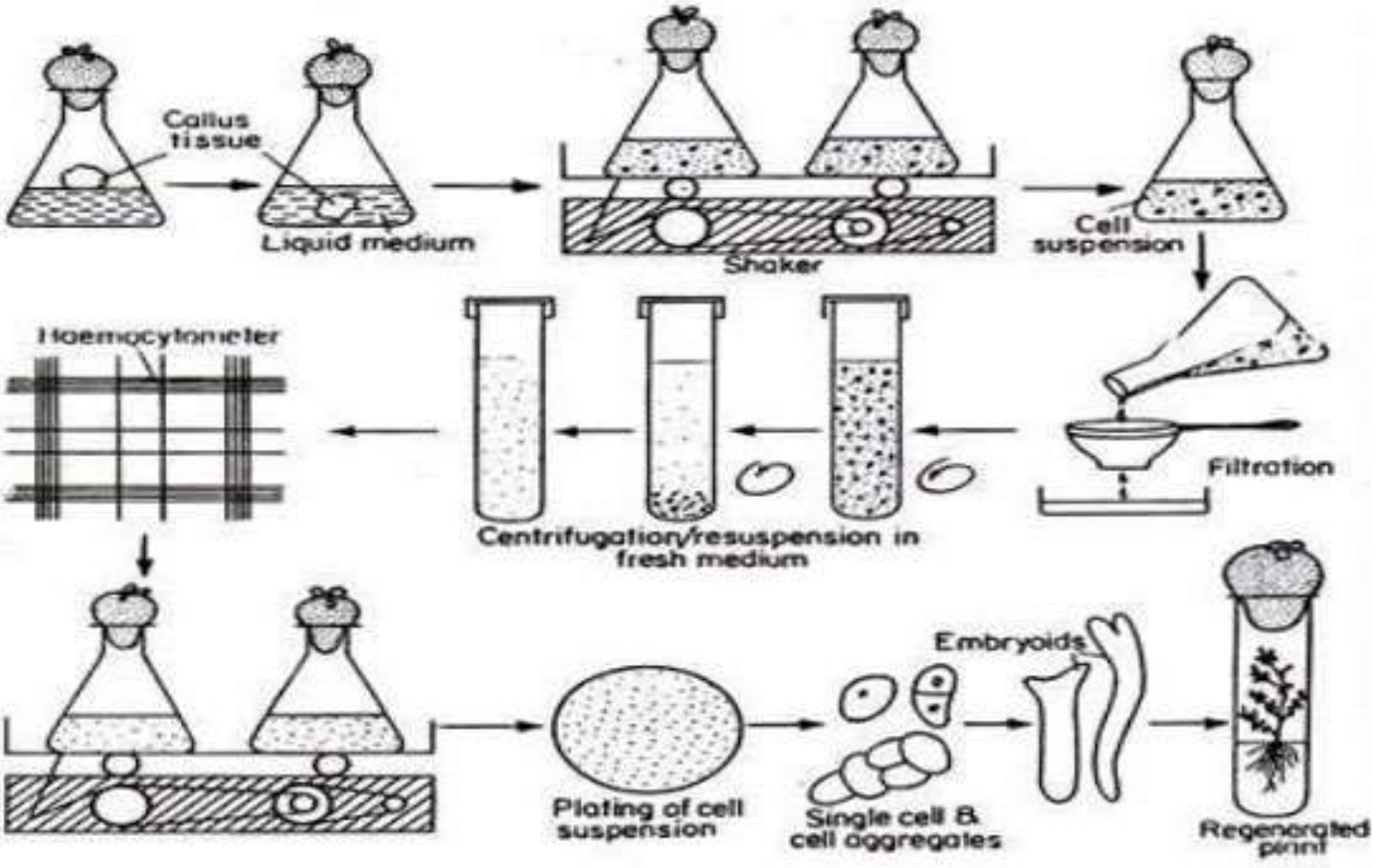
- Incubate at 25°C in dark. Callus start developing within 5-8 days.

- Make histological studies by squashing them in 45% acetic acid, stain by acetocarmic. Use the remaining callus for further studies by subculturing them on same medium in tubes.

Cell suspension-

- Cell suspension is prepared by transferring a fragment of callus (500mg) to the liquid medium (500ml) and agitating the aseptically to make them cells free.
- It is difficult to have suspension of single cell.
- The suspension includes single cell, cell aggregates residual inoculum or dead cells.
- Good suspension consists of a high proportion of single cells than small cluster of cells.
- It is more difficult to have good suspension than to find optimum environmental factors for cell suspension.

CELL SUSPENSION CULTURE



- Kurz and constable have described the properties of cultured plant cell suspensions in common with culture of mocroorganisms as i) They grow in sterile environment ii) They are homogenous in size iii) They can grow in large scale.

Applications of cell suspension cultures-

i). Induction of shoot.

ii). In vitro mutagenesis and selection of mutants.

iii). Genetic transformation studies.

iv). Production of secondary metabolites.

Organogenesis-

- Organogenesis is the adventitious development of non autonomous organs.
- It occurs directly from the explant (direct organogenesis) or indirectly through calli (indirect organogenesis).
- Indirect organogenesis when a shoot as explant is placed on a medium with more cytokinin and less auxin multiple shoot formation occurs.
- Indirect organogenesis occurs when the medium is supplemented with more auxin and less cytokinin leading to callus formation and root development.

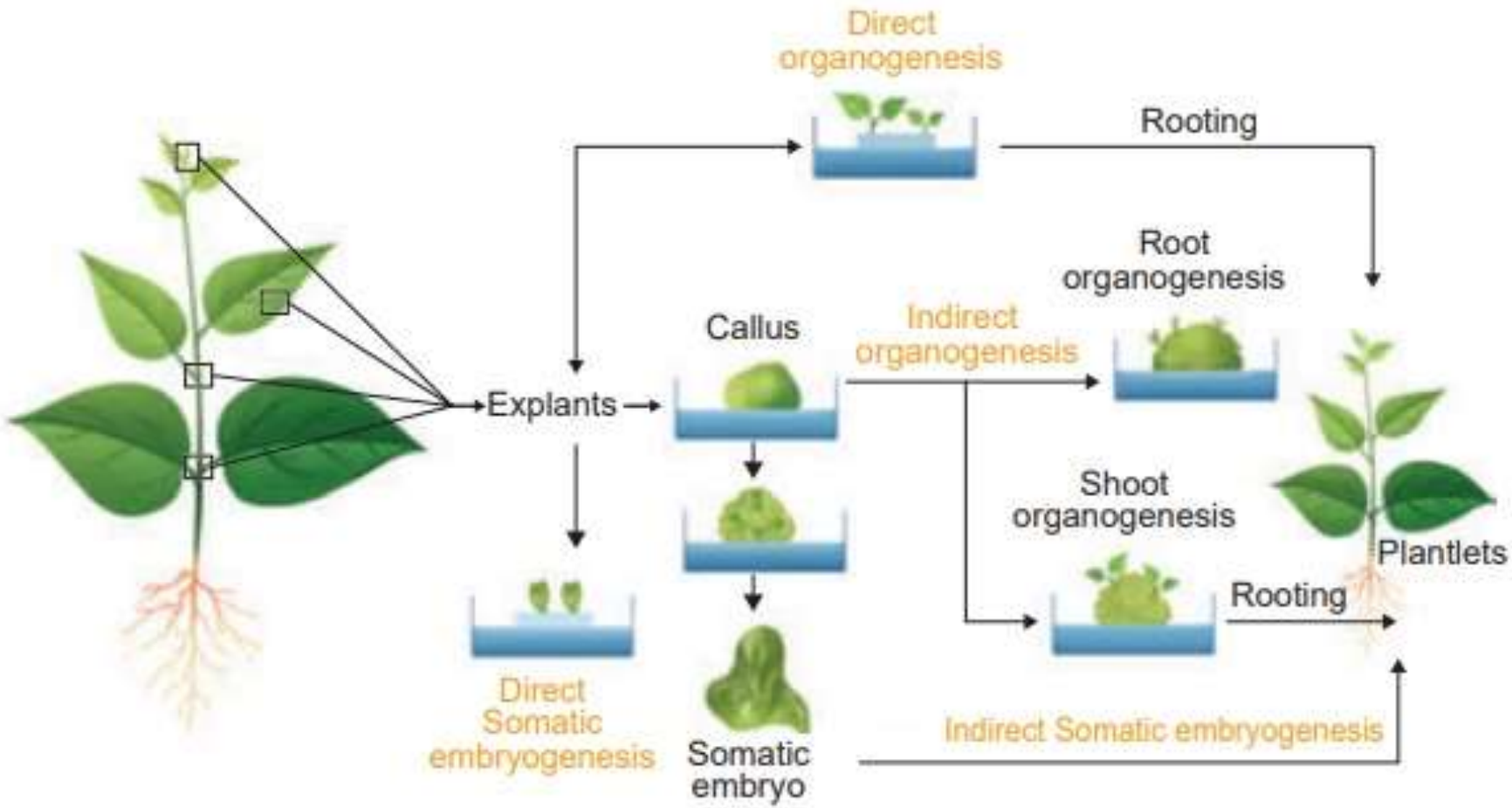


Figure 5.10: Plant Regeneration Pathway

- Depending on the explant 2, 4D at a concentration of 50mg/l will develop callus.

- For development of roots, IBA at 0.1mg/l is used more commonly.

- The external application of growth regulators (auxins and cytokinins) disturbs its original concentration in the explant, leading to shoot or root and callus development.

- The ability of the cells of calli to differentiate into roots and shoots is known as direct organogenesis.

- Each cell is totipotent and is capable of developing into an entire plant, when they are supplied with correct nutrients and environment.

- The process is not dependent on pre-existing initials of roots or shoots. One or few of the cells of a callus give rise to roots and shoots.

- These cells become activated and undergo a series of which lead to the development of a meristemoid.

- This meristemoid is an aggregation of meristem like cells which give rise to either a root or a shoot primordium.

- The manipulation of the ratio of cytokinin to auxin results in shoot formation and high ratio of auxin to cytokinin leads to development of roots.

Requirements-

- Culture media- M.s medium supplemented with growth regulators to raise callus form.
- Explant- *s*prouts of ginger or turmeric.
- Subcultured callus on fresh medium of the growth regulators and their combinations.
- Culture vessels

Sr. No.	Plant sp.	Medium+PGR /M.S	Response obtained
1.	*s*prouts of ginger or turmeric	0.05mg/l BAP 0.50mg/l NAA	

- Wide mouthed and autoclaved100ml flasks used for standard tissue culture with MS medium plus growth regulators as mentioned above.

- Scalpel, inoculating needles, long forceps etc. Disinfectant-1 in 1000HgCl2 or Na hypochlorite and sterile distilled water.

Procedure-

1. Inoculate explants and grow callus.

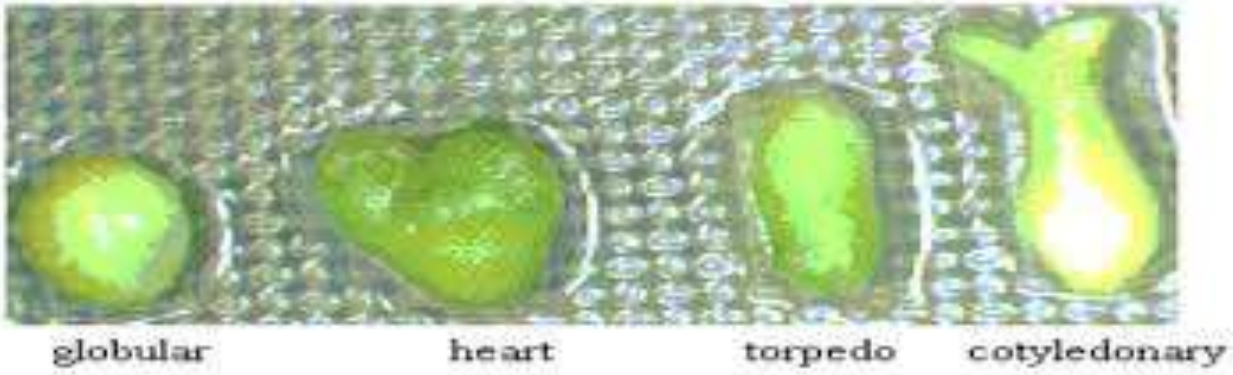
2. Subculture calli to freshly prepared media supplemented with the above cited combinations of growth regulators.

3. Incubate cultures in temperature controlled rooms with flurescent lamps.

4. Observe for organogenesis (root and shoot) medium with 0.05mg/l BAP and 0.50mg/l NAA gives better organogenesis od *s*prouts of ginger or turmeric.

5. Isolate these from callus material and either maintain them or harden them.

Embryogenesis



Shapes of Somatic Embryos in Dicots

Embryogenesis

- Embryos can, not only be produced from fertilized eggs, but can also be produced from somatic tissues that are differentiated by first subjecting it to the action of an auxin and then transferred to a medium without auxin.

- Here it appears that the auxin rejuvenates the cell to such an extent that it requires the ability of a zygote to produce an embryos are known as **somatic embryos**.

- These are diploid embryos, contrast to the haploid embryos that arise from pollen grains .

- Somatic embryos are do not have vascular connection with the mother tissue and hence can easily be separated.

- The first leaves of such embryos will have cotyledonary characters.

- In somatic embryogenesis, as in zygotic embryogenesis, the embryoid passes through globular heart shaped and torpedo shaped stages.

Two types of induction of embryogenesis are there:

1. Pre-embryogenic determined cells (PCDC).
2. Induced embryogenic determined cells (IEDC).

- An embryo has globular, heart shaped and torpedo shaped stages, after which desiccation should occur for it to differentiate.

- This desiccation does not take place in in vitro cultures and hence it has to be induced in order that they develop into normal embroys.

- Desiccation can be induced in vitro by i) ABA ii) Polyethylene glycol (PEG) iii) High concentration of sucrose (reverse Osmosis) iv) Keeping it exposed in laminar clean air flow.

- When callus develops, it may be white, friable, shining or compost light brown coloured one.

- The former one (friable callus) will produce embroys and the latter (light brown) may develop into friable callus at certain points, from where embroys may be produced.

- An immature zygotic embryo when placed on a medium, will germinate and give rise to a plant or give rise to many plants-repetitive embryogenesis.

Requirements-

- Donor plant: *Dacud carrots* (carrot).

- Culture medium: M.S. Medium with and without auxin.

- Few chemical factors have considerable effect on embryogenesis:

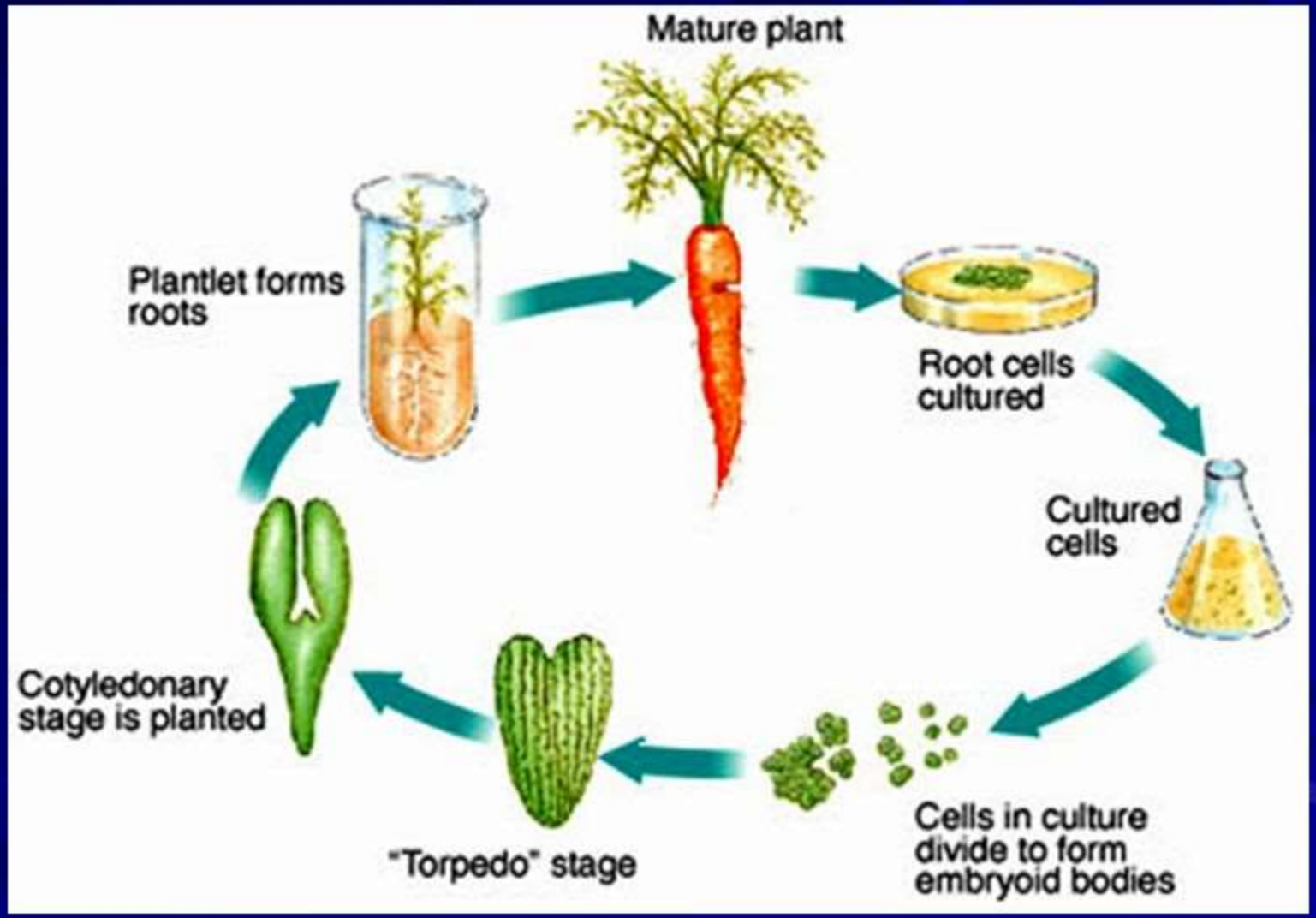
  (i) A substantial amount of reduced nitrogen (NH4) is needed

  (ii) Medium should be supplemented with amino acids like glutamine

  (iii) K+ is the one of that is effective among other ions

  (iv) Auxin should be added at 0.5 or 1.0mg/l of concentration.

- Culture room (temperature 25°C) with 1000lux fluroscent lamps.
- Scalpel, needles, forceps etc.
- Disinfectant, one in 1000HgCl2/ Na hypochlorite and sterile distilled water.
- Filter paper caps or strips.


Mature plant
Plantlet forms roots
Root cells cultured
Cultured cells
Cells in culture divide to form embryoid bodies
"Torpedo" stage
Cotyledonary stage is planted

Procedure-

There are two successive cultures for embryogenesis-

(i) Primary tissue (explant) grown on a medium with auxin where there is no differentiation or embryogenic induction

(ii) Culture of this undifferentiated tissue in an auxin free medium where embryogenesis occurs.

(i)Primary Cultures

- Sterilize pieces of explant in Na hypochlorite solution for 10-15 min and wash 5-6 times with sterile distilled water.

- Trim the ends of this piece of stem with sterile scalpel and cut central piece into bits.

- Place these pieces vertically or horizontally in tubes with medium having auxin and incubate in dark.

- Nodule like proembryonic masses arise. This grow slowly and hence should be transferred after 1½ month.

(ii) Secondary culture-

- Use the same medium without auxin and glutamine to which nodules of primary culture (callus with nodular growth) are transferred and incubated in light.

- Nodules grow into embryos after a few weeks. Embryos will be small heart shaped ones and there will be interwining of these embryos.

- Transfer mature ones to filter paper bridges dipped in liquid M.S. medium at ½ concentration with 5g/l sucrose and incubate in growth chamber.

- When roots develop, transfer to pots with soil mixture.

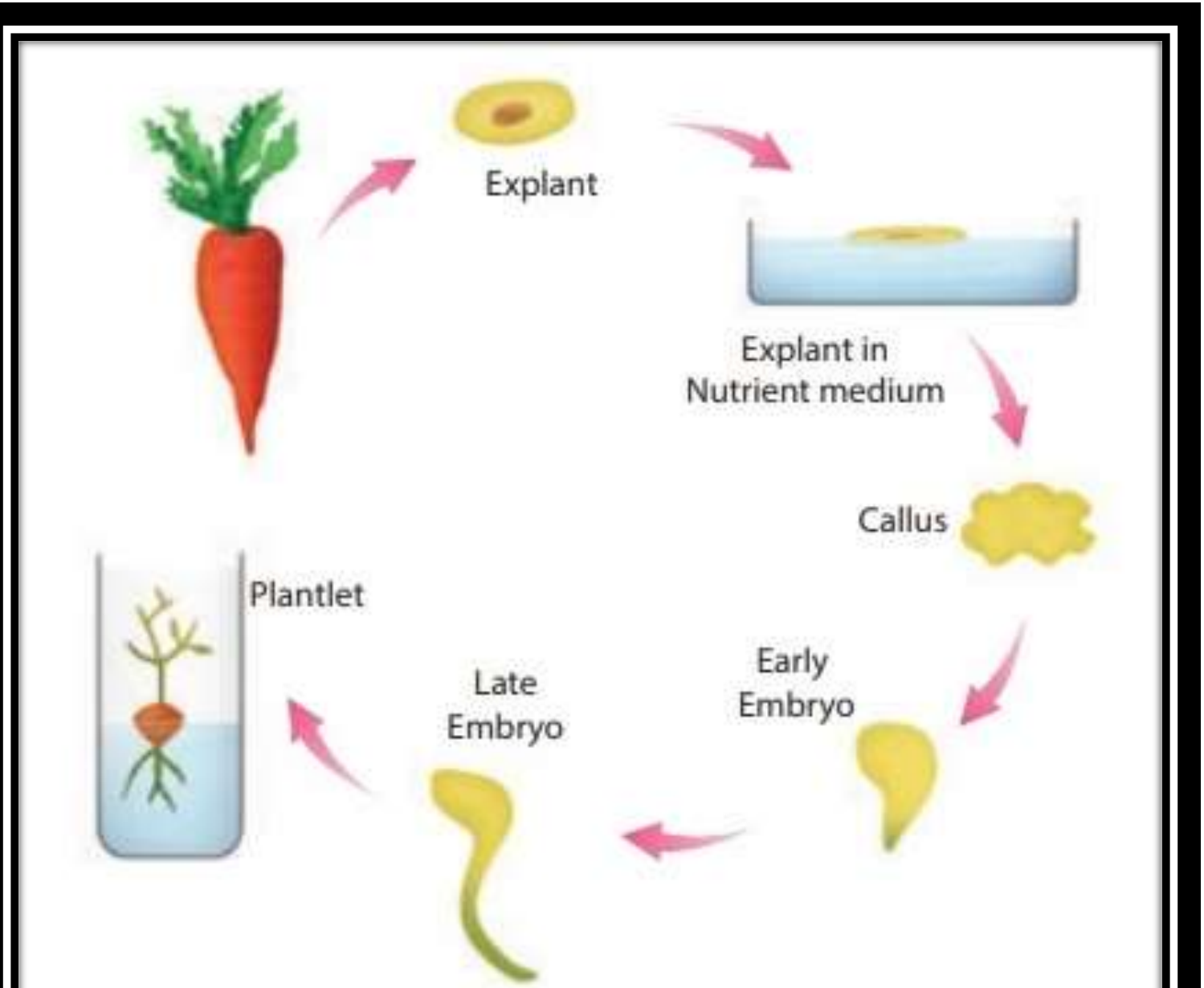


Figure : Totipotency

VIVEKANAND COLLEGE KOLHAPUR (AUTONOMOUS)

Class: B.Sc-I

Topic Name: Study of *Anthoceros*

Name of teacher- Miss. P. B. Shelar

M.sc., MH-SET

- Study of *Anthoceros*- (Hornworts)

- **Classification-**

  Division – Bryophyta

  Class- Anthocerotopsida

  Order- Anthocerotales

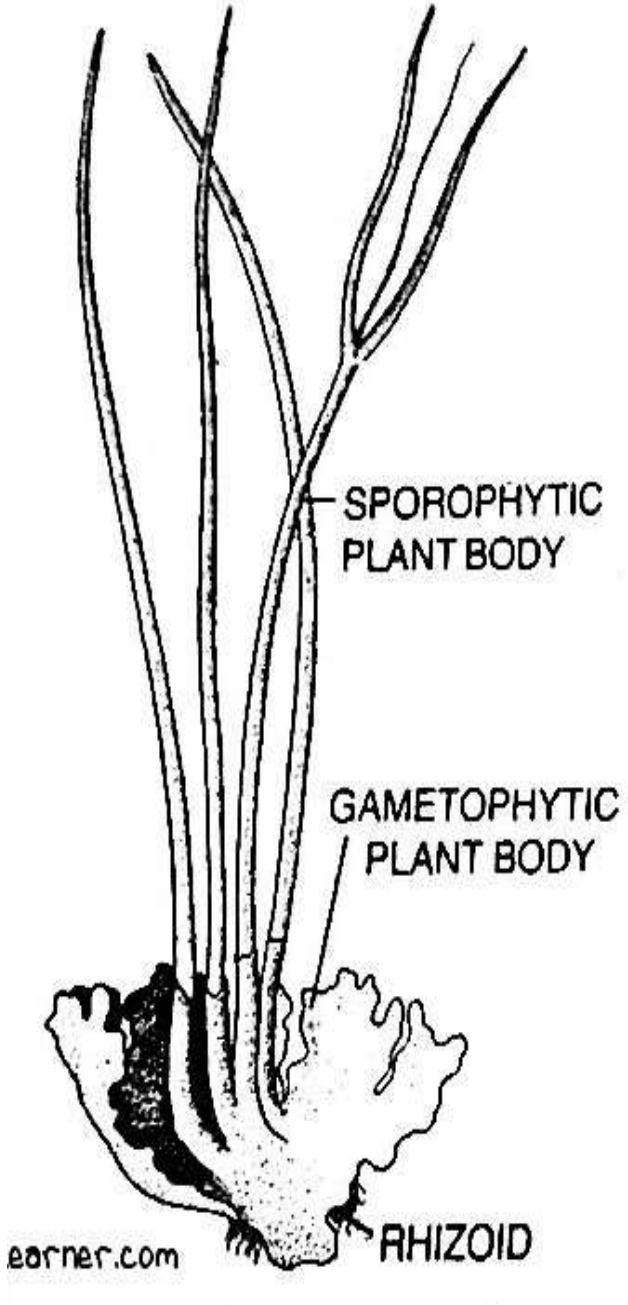
  Family- Anthocerotaceae

  Genus- *Anthoceros*

- **Occurrence-**
  - The plant grows on moist clay soil or the wet rock crevices in moist shady places.

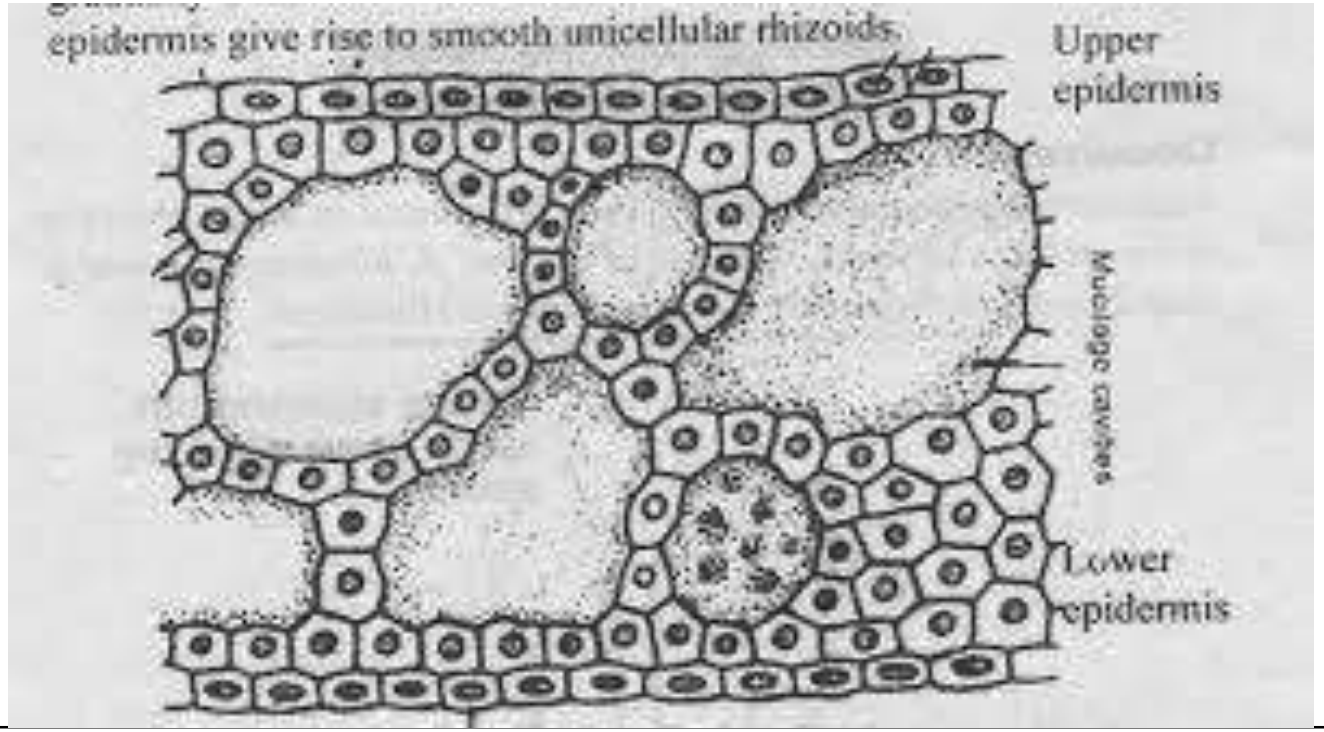
External morphology-

- The gametophytic plant body of Anthoceros is small, dorsiventral, thalloid, prostrate and dark green in colour.
- It displays partial dichotomous branching, giving the thallus a rosette-like appearance.
- Midrib is absent.
- Thallus is not dichotomously branched.
- Dorsal surface smooth, velvety and tough.
- Ventral surface bears smooth walled rhizoids. The ventral surface features unicellular rhizoids that play a crucial role in attaching the thallus to the substratum and absorbing minerals and water from the soil.
- Small colonies of *Nostoc* can also be found on this surface, forming a symbiotic relationship with *Anthoceros* .




© Byrnes Images

- **Anatomy-**
- Internally thallus is homogenus (i.e. all cells are alike). Air pores and air chambers are absent.
- Each cell contains distinct nucleus chloroplast and pyrenoid.
- Parenchymatous tissues lies between upper and lower epidermis.
- On the ventral side there are certain mucilage filled cavities are seen which shows endophytic algae *Nostoc*.



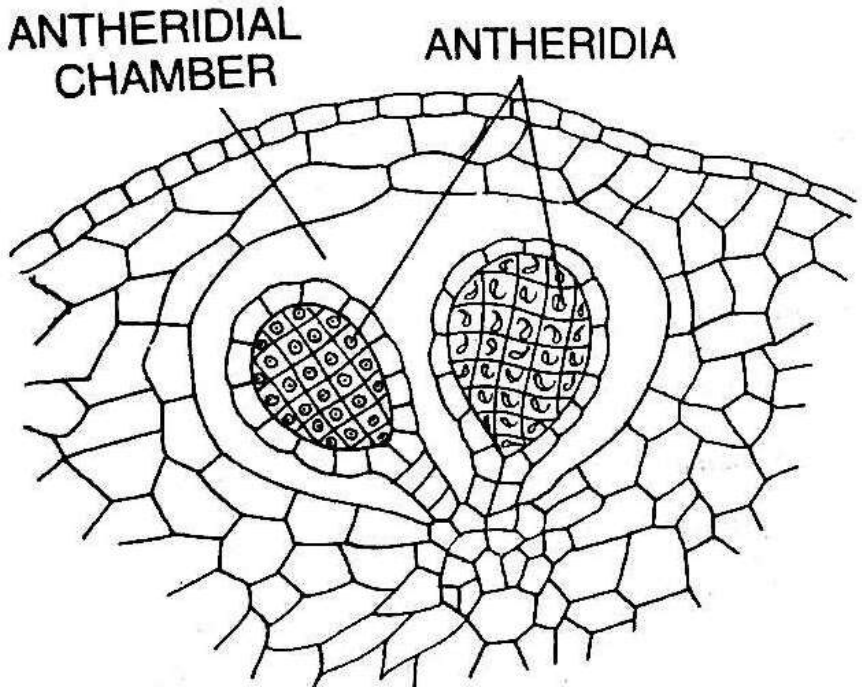
Reproductive structures-

Anthoceros reproduces by vegetative and sexual methods.

I) Vegetative reproduction-

It takes place by formation of Tubers, Gemmae and by progressive death and decay of the older parts of the thallus.

- **Sexual reproduction:**
- Sexual reproduction is oogamous. Male reproductive bodies are known as antheridia and female as archegonia.
- **Antheridium**:
- **Structure:**
    - A mature antheridium has a stalk and club or pouch like body.
    - The stalk attaches the antheridium to the base of the antheridial chamber.
    - Stalk may be slender and composed of four rows of cells or more massive.
    - A single or a group of two to four or more antheridia are present in the same antheridial chamber.
    - A single layered sterile jacket encloses the mass of androcytes which metamorphosis into antherozoids.



- **Archegonia-**
- Archegonia develop in the flesh of the thallus on dorsal surface. The place of an archegonium on a thallus can be identified by the presence of a mucilage mound.
- **Structure:**
- A mature archegonium consists of two to four cover cells, an axial row of four to six neck canal cells, a venter canal cell and an egg. The jacket layer is not distinct from the other vegetative cells like other Bryophytes.

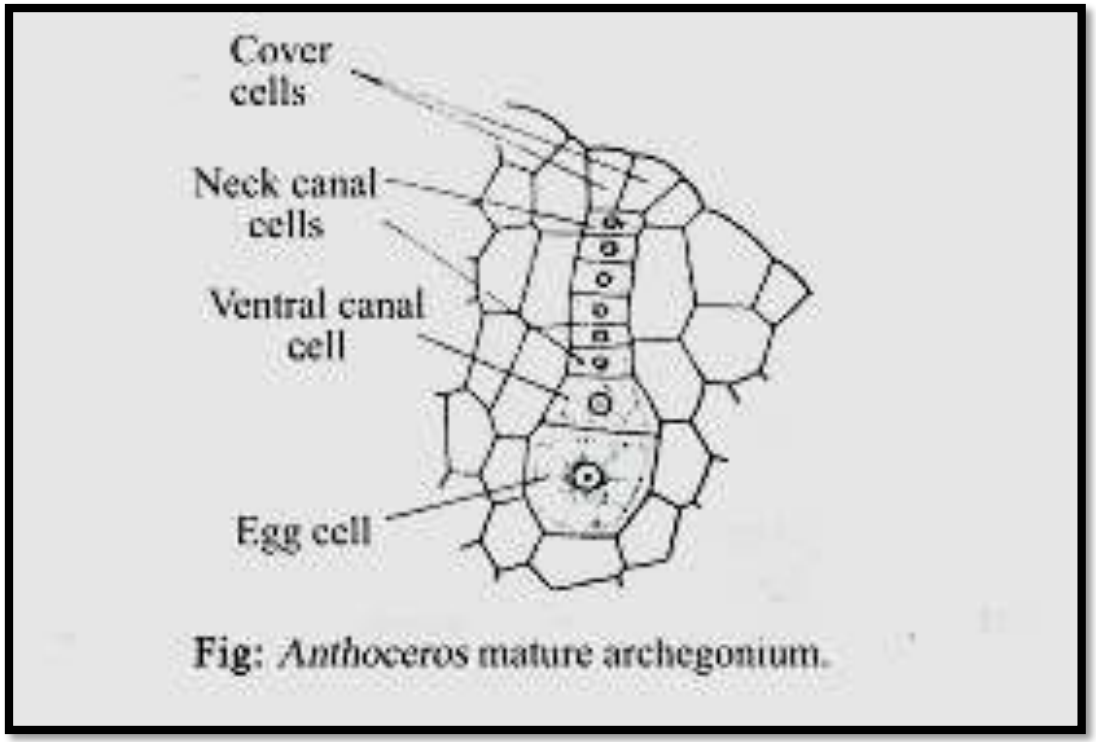


Fig: *Anthoceros* mature archegonium.

- **Fertilization:-**
- Water is essential for fertilization.
- In the mature archegonium, the venter canal cell, neck canal cells disintegrate and form a mucilaginous mass.
- It absorbs water, swells up and becomes out of the archegonial neck by pushing the cover cells apart.
- This mucilaginous mass becomes continuous with the mucilage mound and in this way an open passage down to egg is formed.
- The mucilaginous mass consists of chemical substances.
- Many antherozoids caught in the mucilage enter in the archegonial neck because of the chemotactic response, reach upto the egg, and fertilization is effected.
- Prior to fertilization, egg enlarges and fills the cavity of the venter. Fusion of both male and female nuclei results in the formation of diploid zygote or oospore.
- Fertilization ends the gametophytic phase.

- **Sporophytic Phase:**
- **Structure of Mature Sporogonium:**
- The mature sporophyte consist a bulbous foot and a smooth, slender, erect, cylindrical, structure called **capsule**. Capsule varies in length from two to fifteen centimeter in different species. The sporogonium appears like a **'bristle'** or **'horn'**, hence, the species are called '**hornwoits'.**

Internal structure:-

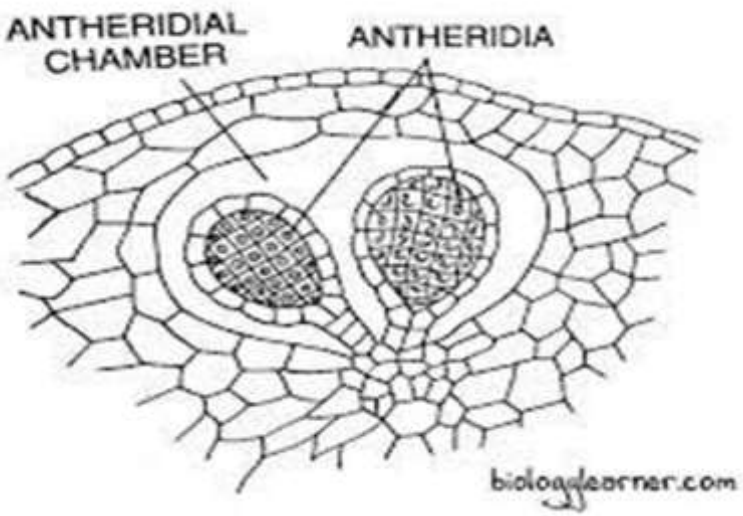
- A mature sporogonium can be differentiated into three parts viz., the foot: seta and the capsule.

Foot:

- It is bulbous, multicellular and made up of a mass of parenchymatous cells. It acts as ac haustorium and absorbs food and water from the adjoining gametophytic cells for the developing sporophyte.
- Seta is represented by meristematic zone. This is present at the base of the capsule and consists meristematic cells. These cells constantly add new cells to the capsule at its base.
- The presence of meristem at the base enables the capsule to grow for a long period and form spores. It is a unique feature of Anthoceros and is not found in any other bryophyte. We are able to see different stages of development from base upwards in the sporogonium of Anthoceros.

II) Sexual reproduction-

The **male** sex organ (**Antheridium**) and **female** sex organ (**Archegonium**) are embedded in dorsal surface of thallus.



a) Antheridium-

- The antheridia are produced in antheridial chamber on the dorsal surface of thallus.
- The mature antheridium is oval, stalked, multicellular surrounded by single layered jacket.
- Inside the jacket there are many androcytes.

b) Archegonium-

- The archegonia are produced on dorsal surface of thallus.
- The archegonium consist of elongated neck canal cells and swollen venter canal cells.
- Mature archegonium consist of 4-6 neck canal cells, One venter canal cell and one egg cell.
- At the time of fertilization neck canal cell degenerates and antherozoid fuses with egg to form zygote.

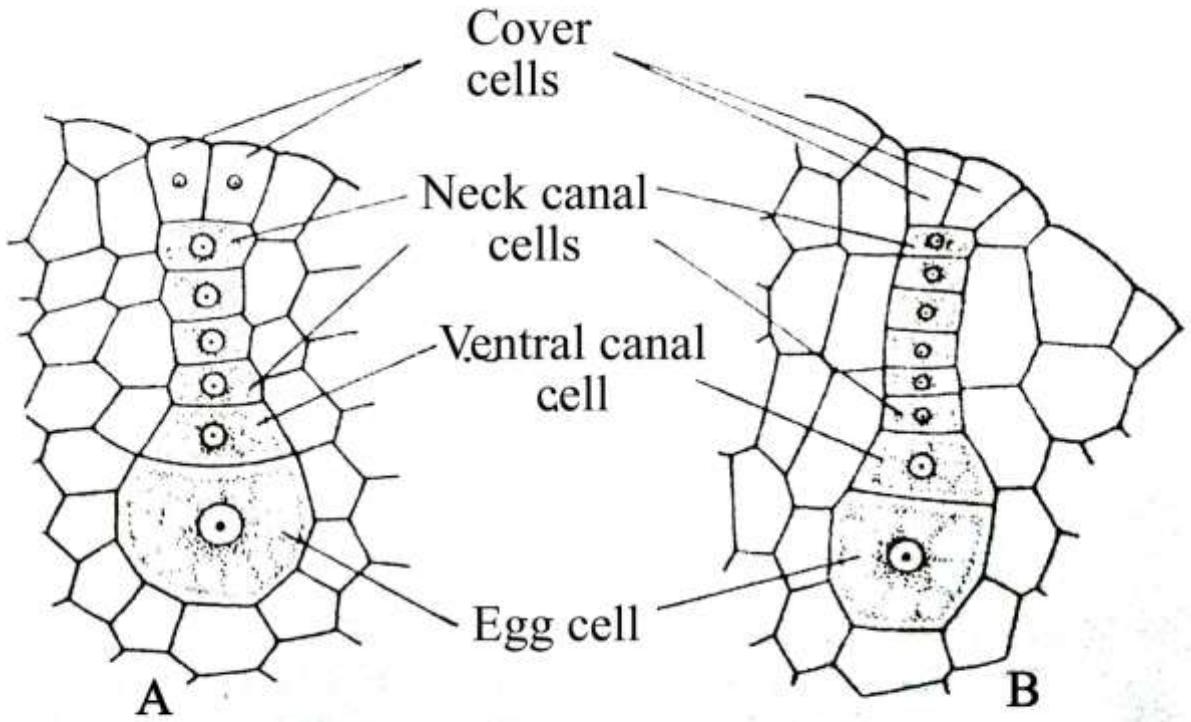
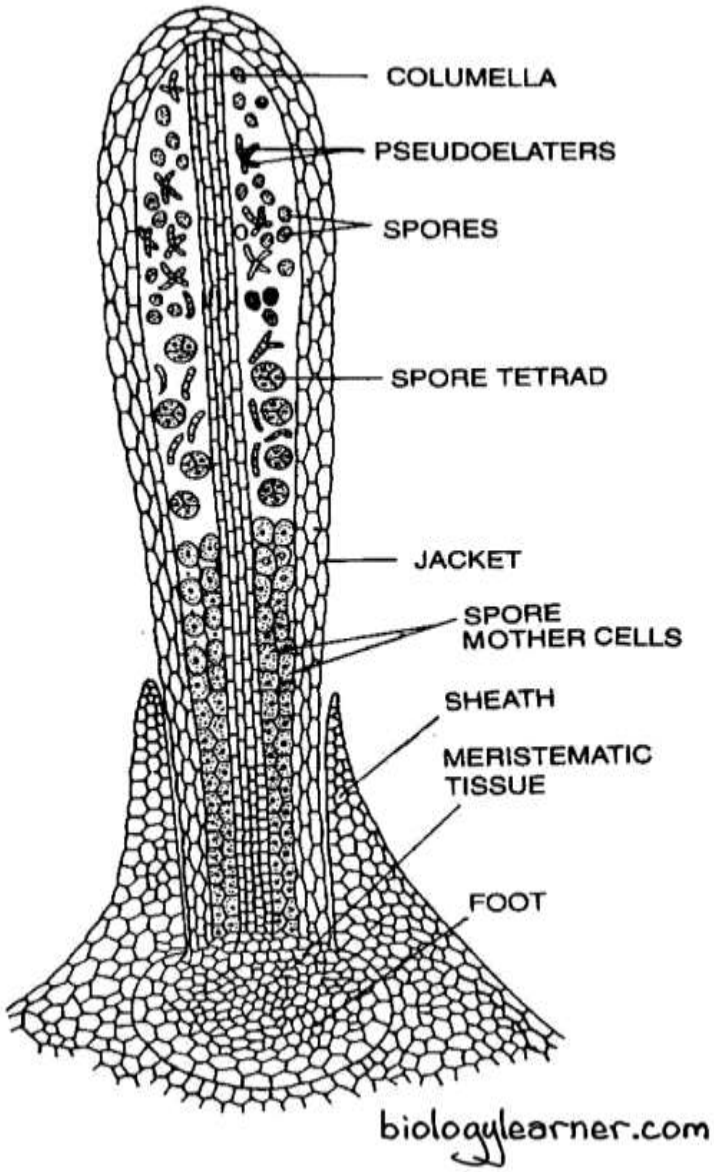


Fig: *Anthoceros.* (A-B) Nearly mature archegonium.

- **Sporophyte**-
  - The sporophyte are linear, long elongated structures arising from the dorsal surface of the thallus.
  - It is differentiated with bulbous foot and long capsule
  - Foot is embedded in the gametophyte.
  - At the center columella is present it is made up of 16 vertical rows of cell extending from the base to the apex.
  - At the base of the capsule single to double layered archesporium is present. In the middle part of the capsule spore mother cells are present and in upper region spore tetrads and pseudo-elators are present.
  - At the upper most region of the capsule separated spores and pseudoelators are seen.
  - The wall of the capsule 4-6 layered.
  - The capsule shows longitudinal dehiscence from the tip region from which mature spores are dispersed.



VIVEKANAND COLLEGE KOLHAPUR (AUTONOMOUS)

Class: B.Sc-II

Topic Name: Types of Ovules

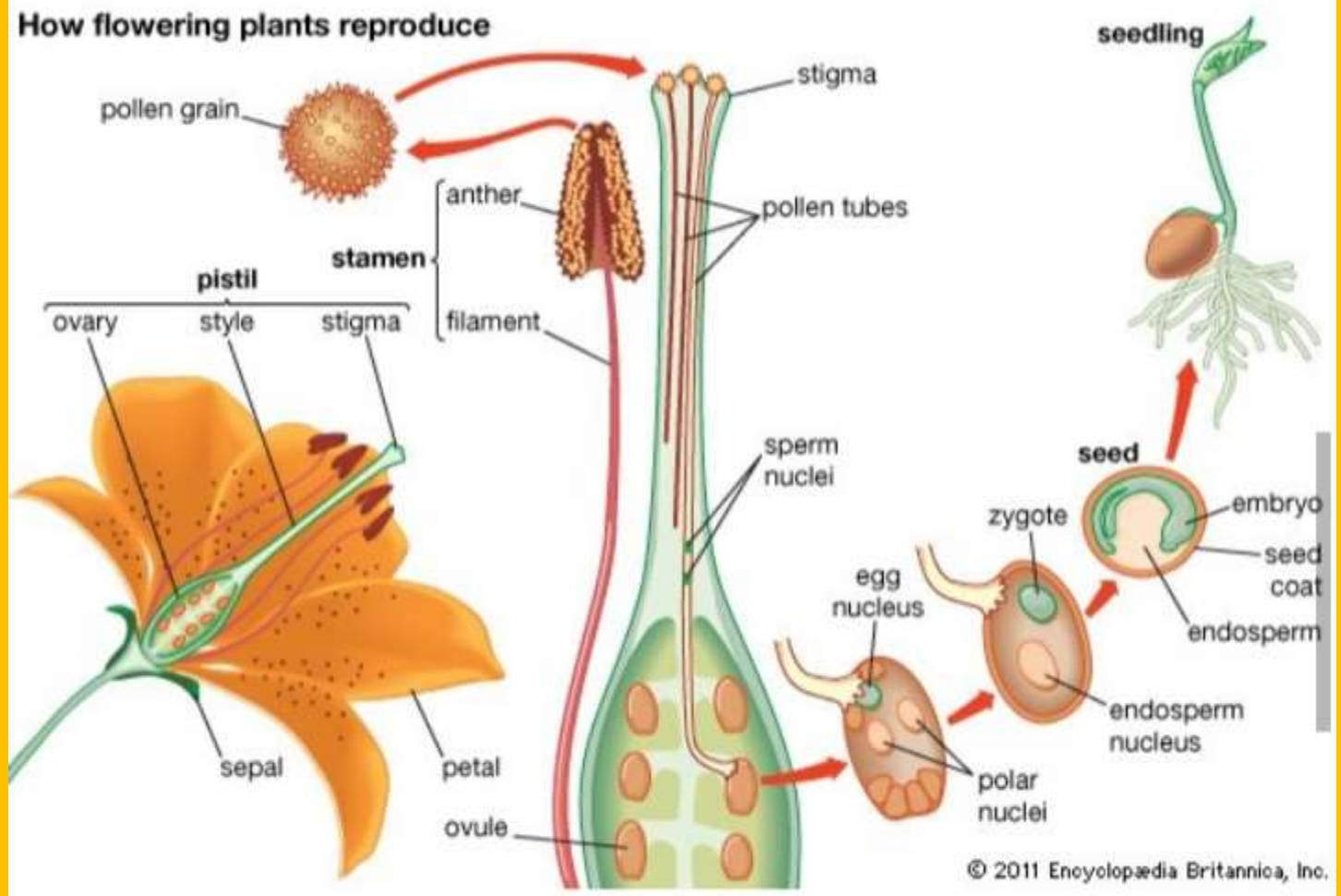
Name of teacher- Miss. P. B. Shelar

Study of types of Ovule

- In flowering plants, the ovule is the structure that gives rise to and contains the female reproductive cells. It consists of three parts: the integument- forming its outer layer, the nucellus and the female gametophyte in its center.

- The female gametophyte known as megagametophyte is also called as embryo sac in angiosperms.

- The mega gametophyte produces an egg cell for the purpose of fertilization.


How flowering plants reproduce
pollen grain
stigma
anther
pollen tubes
stamen
pistil
ovary
style
stigma
filament
sperm nuclei
seedling
seed
embryo
zygote
seed coat
egg nucleus
endosperm
endosperm nucleus
sepal
petal
polar nuclei
ovule
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- Micropyle - A minute opening in the integument of on ovule of a seed plant.
- Nucellus - It is the central part of a plant ovule that encloses the female gametophyte.
- Funicle – A stalk, that attaches an ovule to the placenta in the ovary of flowering plant.
- Chalaza - Basal part of the ovule.
- Integument – One or more protective envelopes around the ovule.
- Raphae – Longitudinal ridge on the side of certain ovules or seeds.
- Hilum – It is a junction between ovule and funicle

Practical I –Practical no 15
Study of Typical flower and its Parts

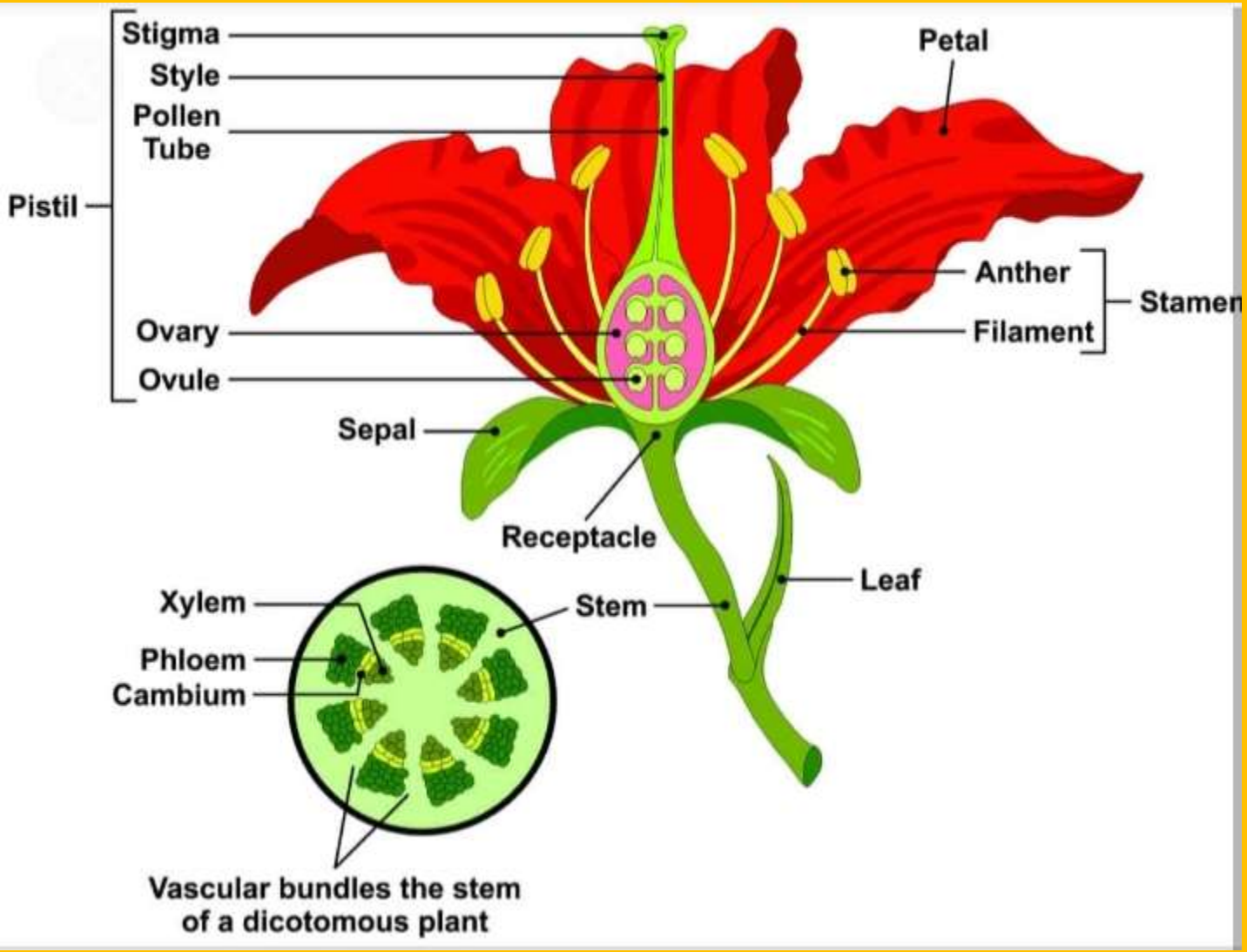
- by Dr Alvikar A. R

- The seed bearing part of a plant, consisting of reproductive organs (Stamens, and carpel's) that are typically surrounded by brightly colored corolla (Petals) and a green calyx (Sepals).

- The sole purpose of flowers is sexual reproduction, therefore ensuring the survival of the species.

- Many flowers that rely on pollinators, such s birds and butterflies, have evolved to have brightly colored petals and appealing scents s a way to attract the attention of the pollinators.

Parts of Flower



Parts of flowers

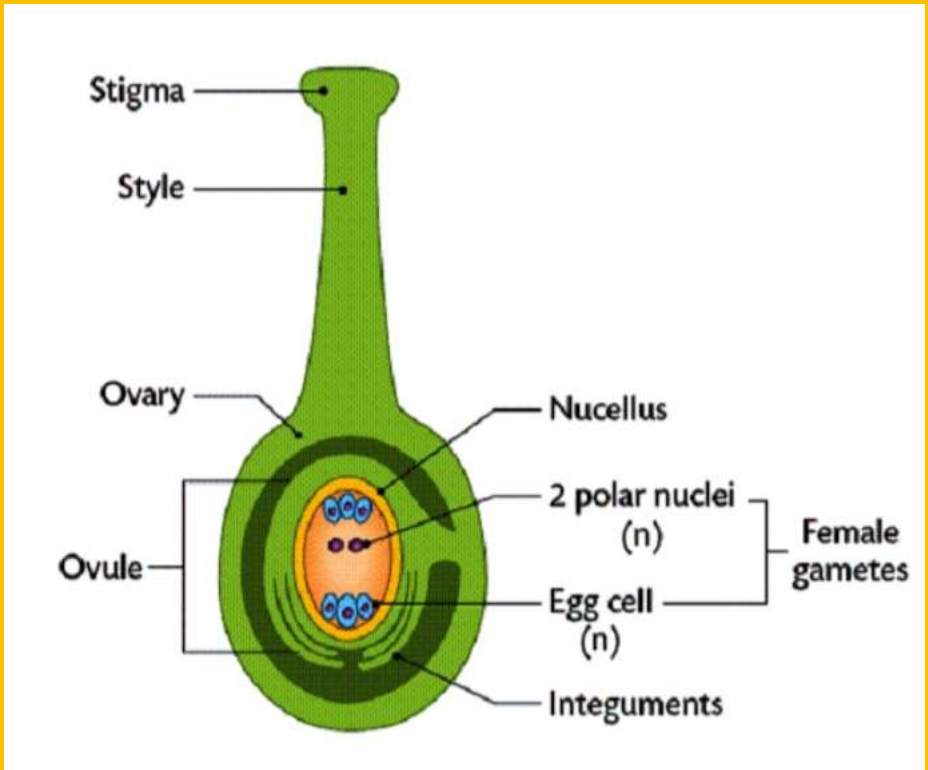
- Petals (Corolla) – Petals are modified leaves that surround the reproductive parts of flower, they are brightly colored or usually shaped to attract pollinators.

- Sepal (Calyx) - The outer green, leaf like part of flower protects the developing bud.

- Peduncle – This is the stalk of the flower.

- Receptacle - It is thickened part of a flower stalk where the parts of the flower are attached.

Female reproductive part of flower and its function



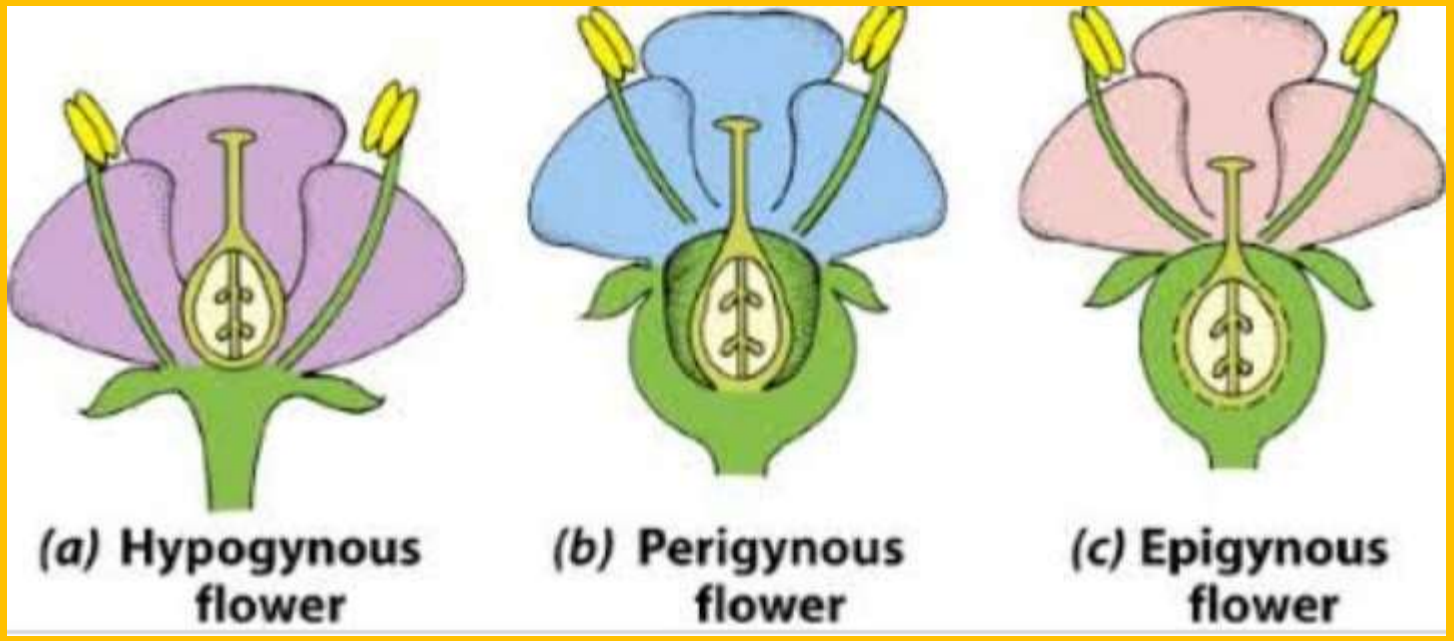
Carpel (Pistil) – It is flask shaped, female reproductive part of a flower. It contain main three parts. A collection of pistil is called gynoecium.

1. Stigma – It receives the pollen during fertilization.

2. Style – It is long elongated stalk of the pistil which is present above the ovary.

3. Ovary – It contains the ovules, it is the part of the plant where the seed formation takes place.

4. Ovules – These are reproductive cells which will become the seed when fertilized by pollen.

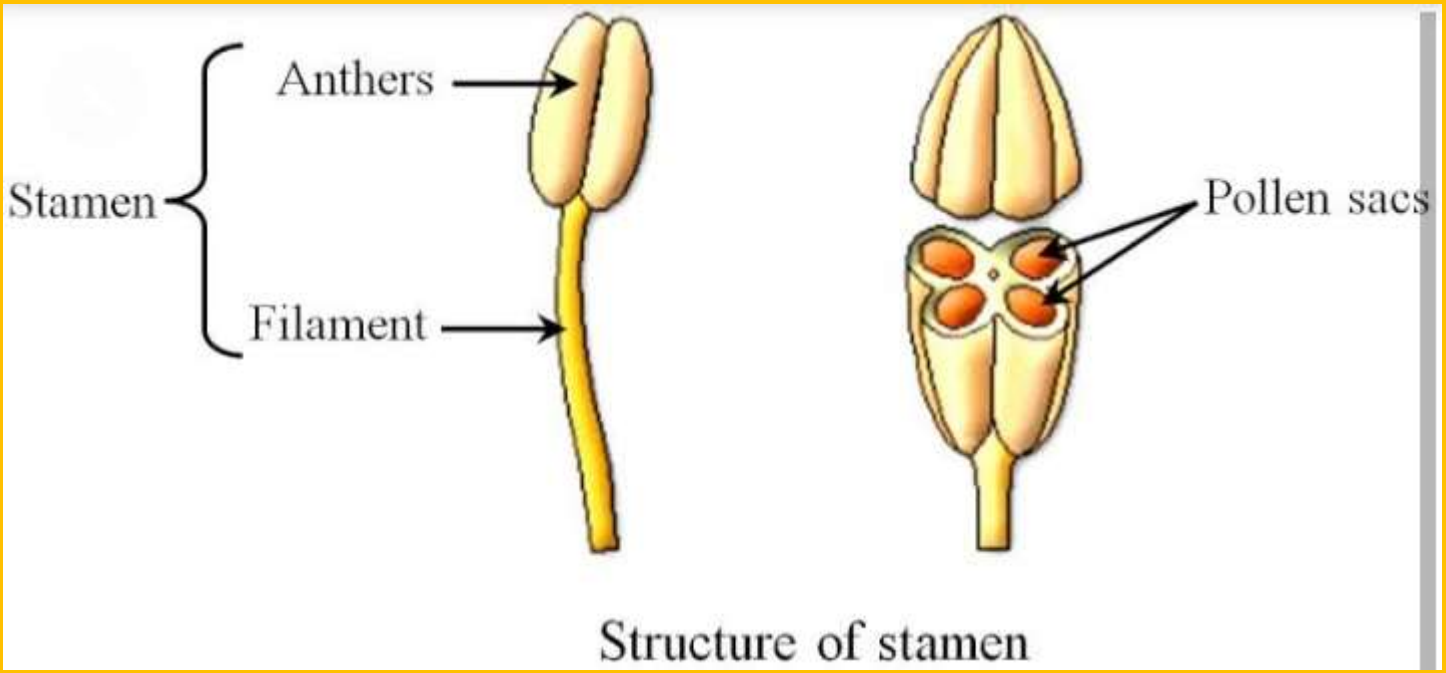


❖ Hypogynous ovary – The flower is said to be hypogynous if sepals, petals and stamens are inserted below the ovary.

❖ Perigynous ovary - The thalamus forms a cup-shaped structure around the ovary, and sepals, petals and stamens appear or attached to the rim of the hypanthium.

❖ Epigynous flower – If the sepals , petals and stamens in a flower arise from the top of the ovary then it called as epigynous flower.

Male Reproductive part of a flower



Stamens are the male parts of the flower. Many stamens are collectively known as the androecium. They are structurally devided in to twp parts.

1. Anthers – It is head of the stamen and is responsible for producing the pollens.

2. Filament – It is long and slender part attached to the anther to the flower.

- Micropyle - A minute opening in the integument of on ovule of a seed plant.

- Nucellus - It is the central part of a plant ovule that encloses the female gametophyte.

- Funicle – A stalk, that attaches an ovule to the placenta in the ovary of flowering plant.

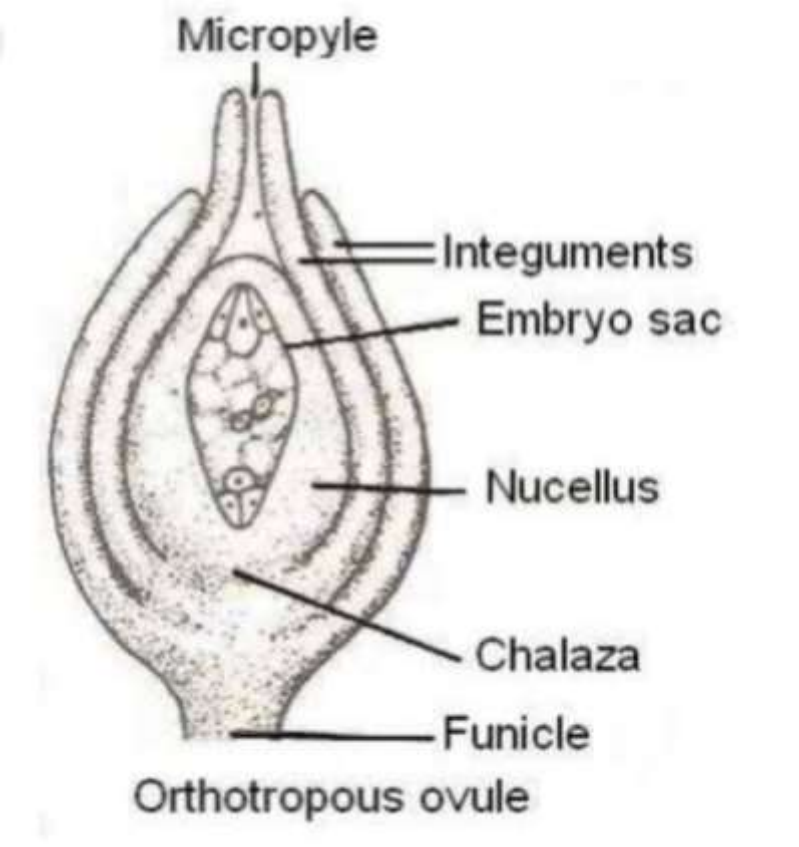
- Chalaza - Basal part of the ovule.

- Integument – One or more protective envelopes around the ovule.

- Raphae – Longitudinal ridge on the side of certain ovules or seeds.

- Hilum – It is a junction between ovule and funicle

❖ Ovule is integumanted megasporangium it encloses embryo sac which is the female gametophyte of angiospersms, depending up on the shape and orientation, the ovule are classified into five types.



1. **Orthotropous ovule**

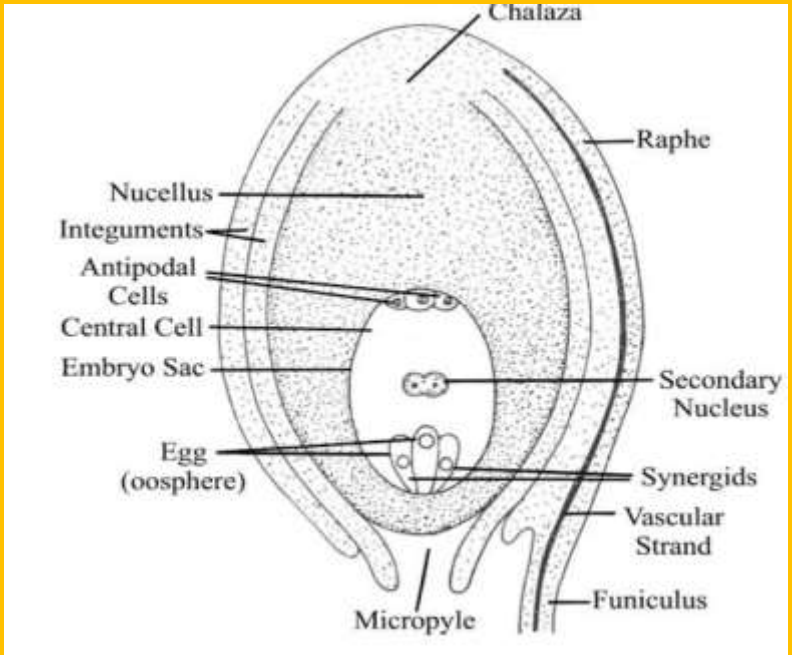
❖ The body of the ovule is erect or straight.

❖ The hilum, chalaza and the micropyle lie in a straight line.

❖ In which no curvature takes place during development.

❖ The micropyle is positioned opposite the funiculus base.
e.g Polygonum

2. Anatropous ovule

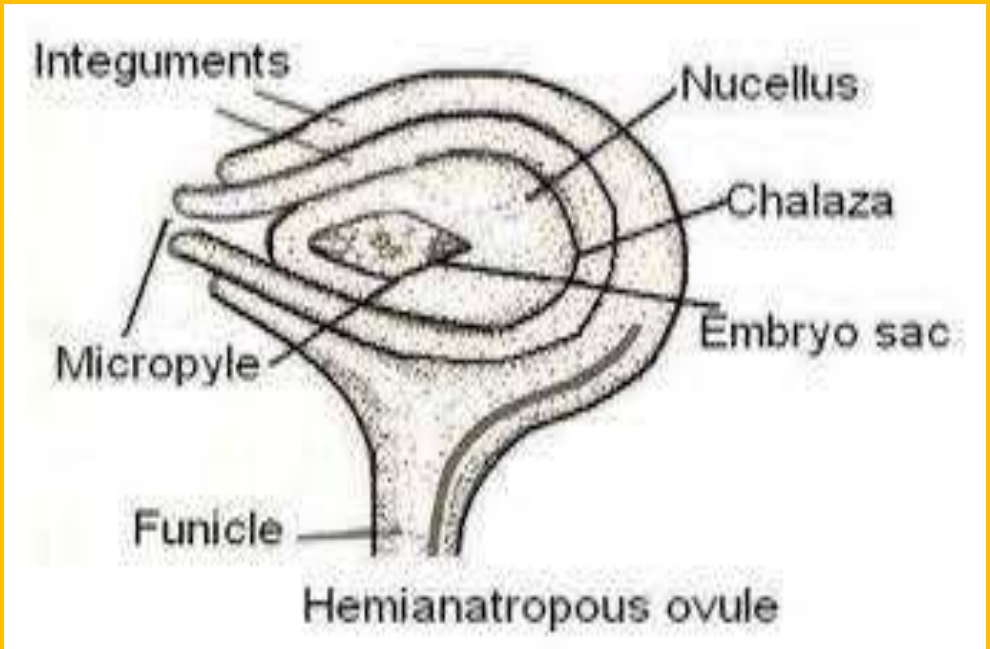


❖ The body of the ovule becomes completely inverted during the development so that the micropyle lies very close to the hilum.

❖ The ovule is completely inverted in its orientation, due to curvature of the funicule.

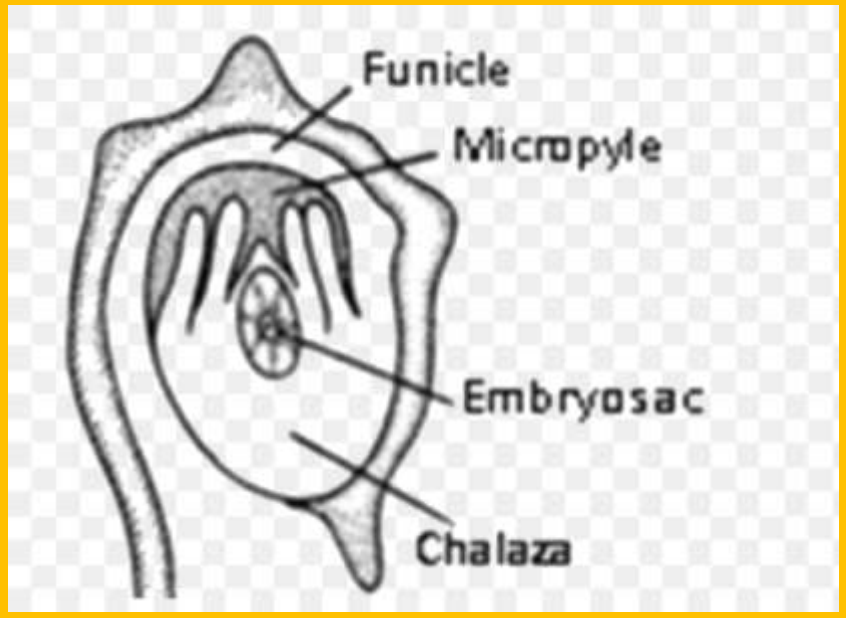
❖ Longitudinal axis of the nucellus is parallel to funicule axis.
e.g. Gamopetalae members

3. Hemianatropous ovule



- In this type the nucellus and integuments lie more or less at right angles or 90 degrees to the funicle.
- It is also known as transverse ovule.
- E.g. Rananculus

4. Circinotropous ovule

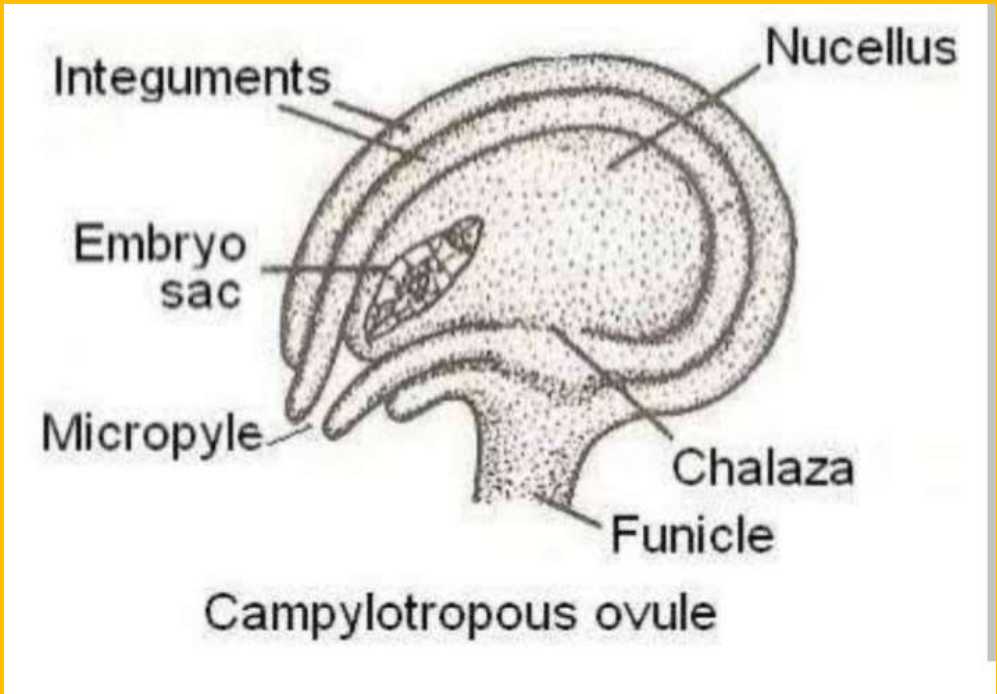


❖In this type of ovule the length of the funiculus increased and it covers the whole ovule.

❖ The nucellus and the axis are in the same line in beginning but due to rapid growth on one side, the ovule becomes anatropous.

❖ The curvature continues further and the micropyle again points upwards. E.g Opuntia.

5. Campylotropous ovule

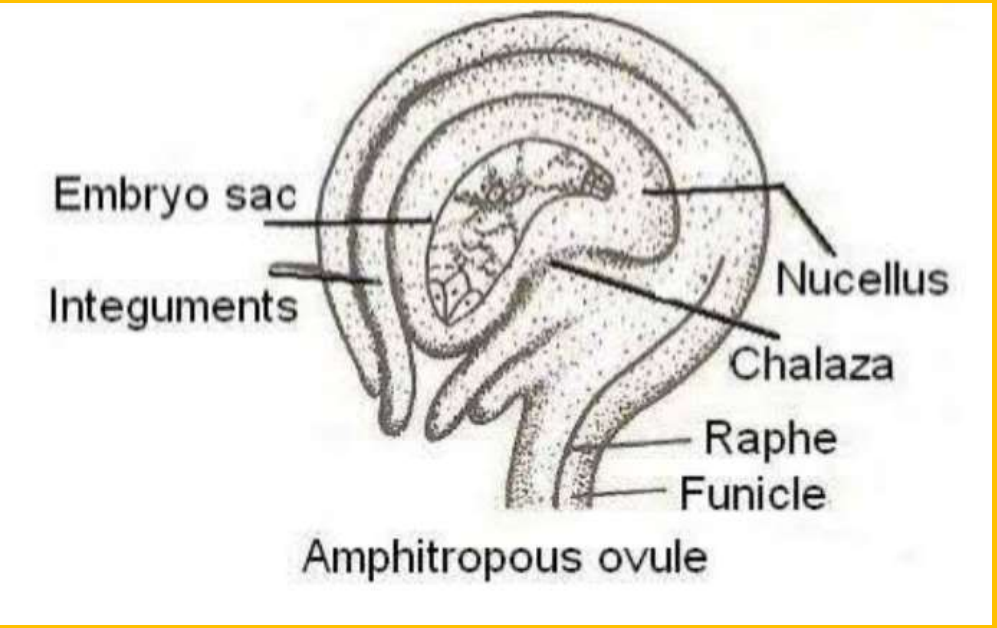


❖ The body of the ovule is curved or bent round so that the micropyle and chalaza do not lie in the same straight line. E.g. Leguminosae

❖ The micropyle is directed towards the base of the funicle because of the curvature of the nucellus.

❖ The funicle is attached near middle the body of the ovule. E.g Mustard, Capparis etc.

6. Amphitropous ovule

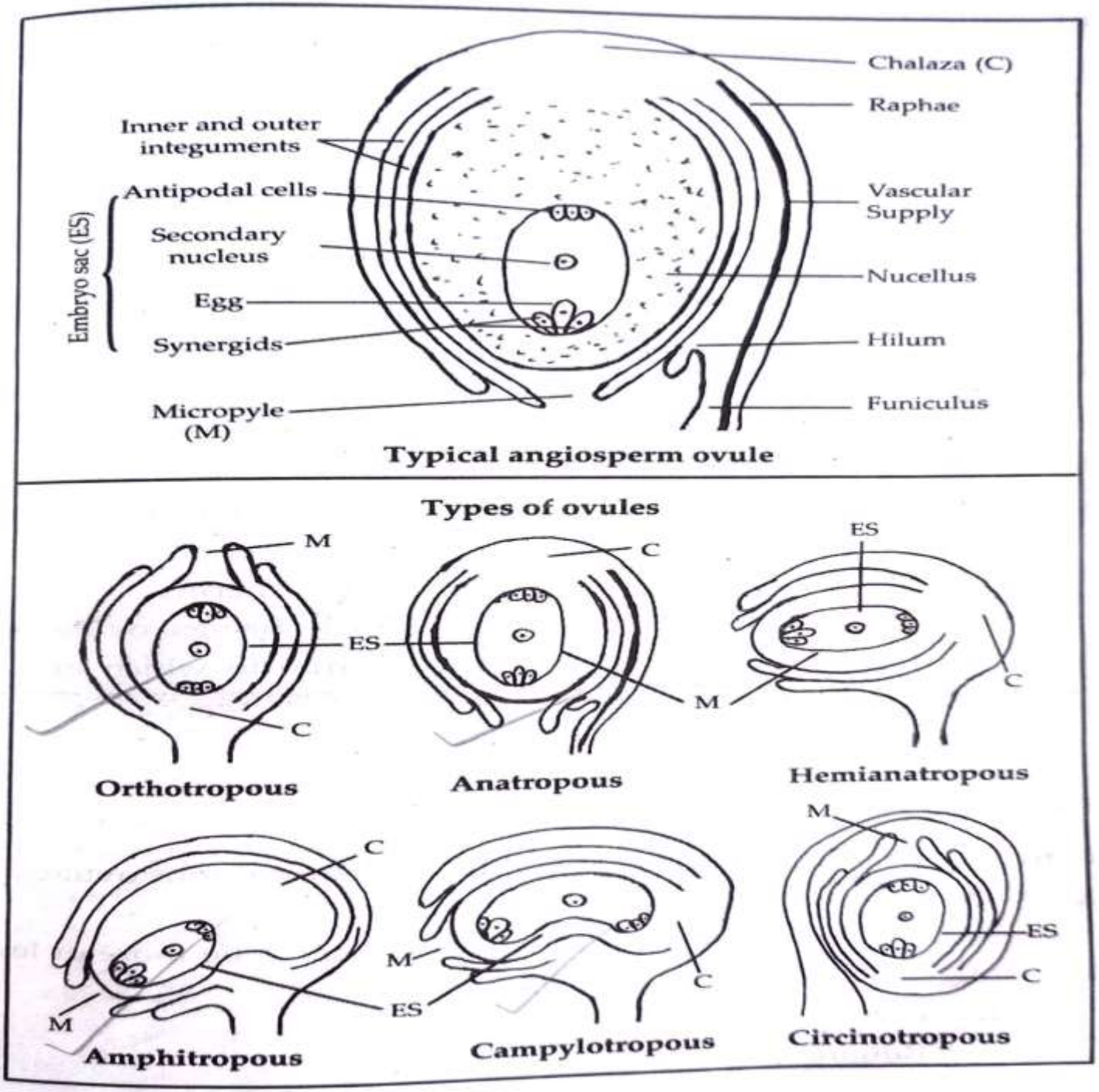


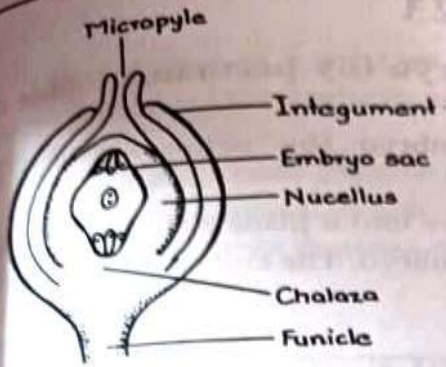
❖ The curvature of the ovule is very much pronounced and the embryo sac also becomes curved.

❖ The embryo sac is also curved like horse-shoe shape.

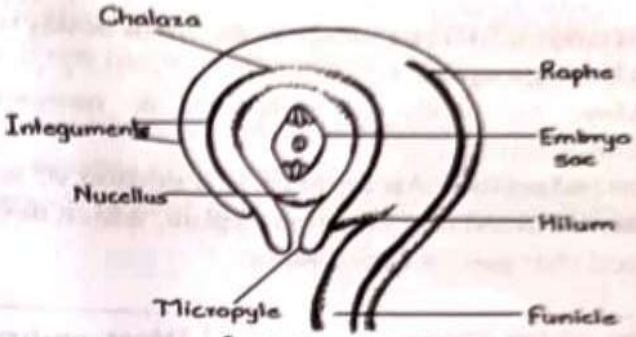
❖ The funicle is attached near the middle of the body.

❖ The micropyle, chalaza and hilum come close to each other. E.g Clematis

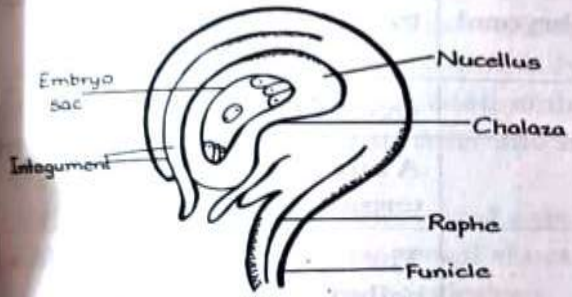

Chalaza (C)
Raphae
Inner and outer integuments
Antipodal cells
Vascular Supply
Embryo sac (ES)
Secondary nucleus
Nucellus
Egg
Hilum
Synergids
Micropyle (M)
Funiculus
Typical angiosperm ovule
Types of ovules
M
ES
C
Orthotropous
Anatropous
Hemianatropous
Amphitropous
Campylotropous
Circinotropous



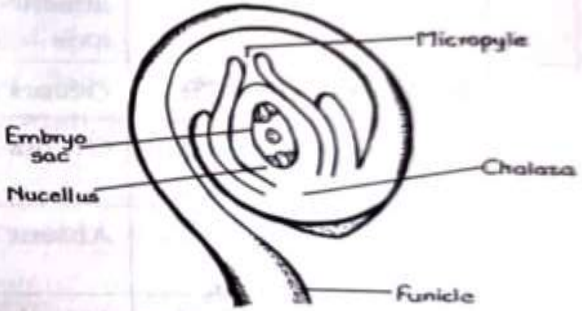
Orthotropous ovule.



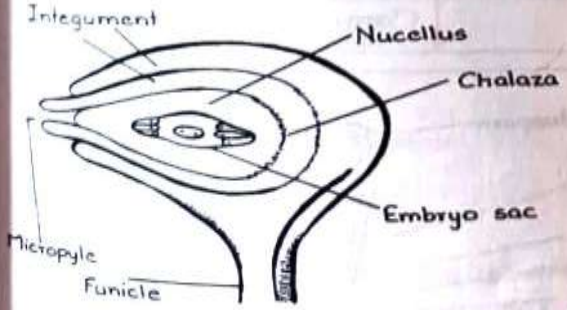
Anatropous ovule.



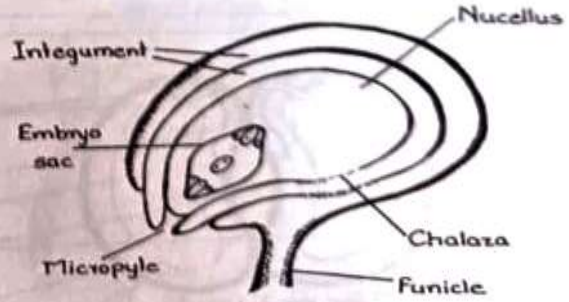
Amphitropous ovule.



Circinotropous ovule.



Hemianatropous ovule



Campylotropous ovule