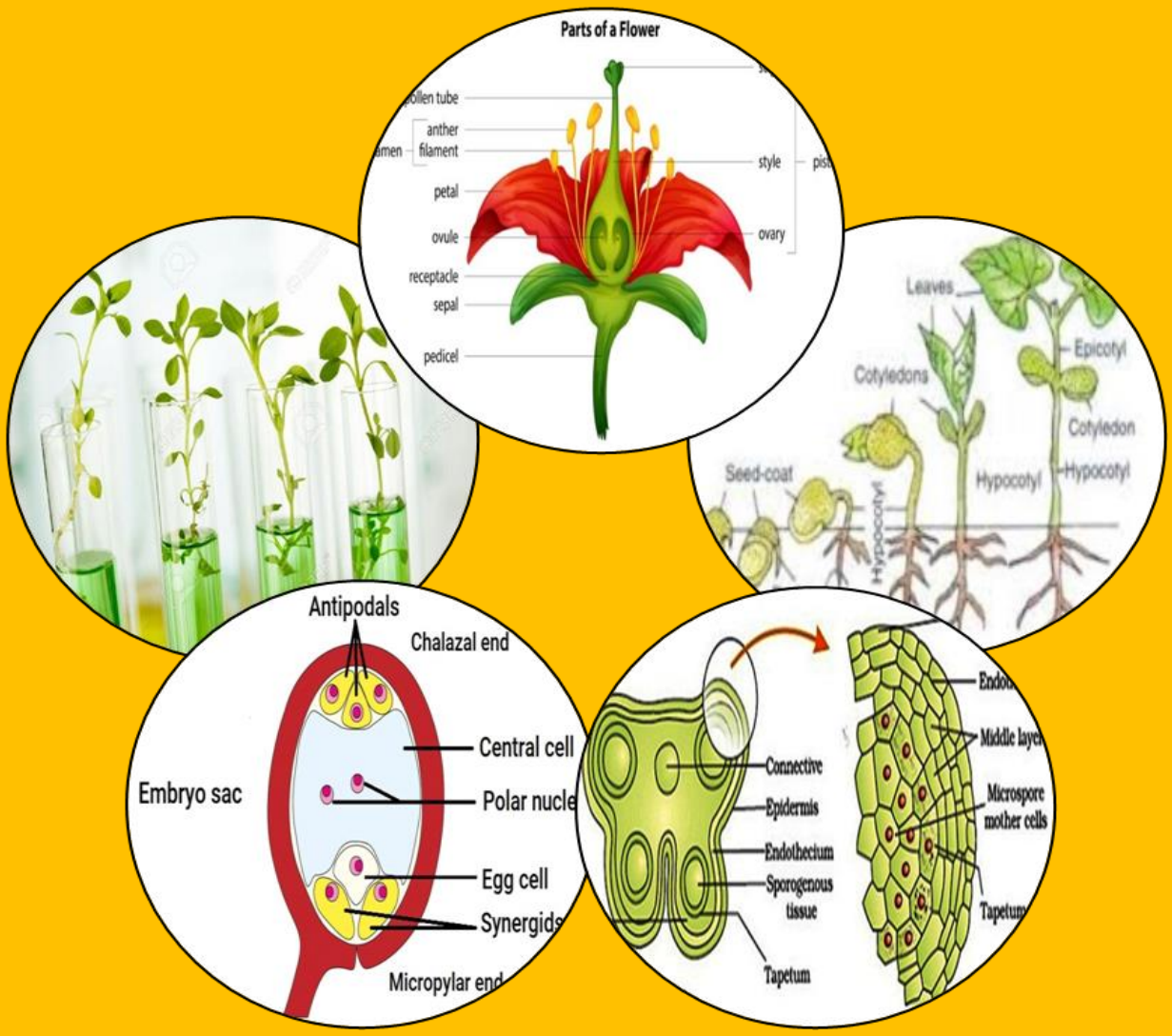
MSCBOT-509

M. Sc. II Semester

PLANT REPRODUCTION



DEPARTMENT OF BOTANY

SCHOOL OF SCIENCES

UTTRAKHAND OPEN UNIVERSITY

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UNIT-1: THE FLOWER: A MODIFIED SHOOT, PARTS AND TYPES OF FLOWERS

1.1 OBJECTIVES

After reading this unit students will be able:

- To know that the flower is a modified shoot
- To learn about the different parts of flower
- To understand about different types of flower
- To know about the different types of reproduction

1.2 INTRODUCTION

A **flower** is a **modified shoot** (**stem** bearing leaves). In a typical f**lower** the **stem portion** is condensed to form a compact receptacle called thalamus. The thalamus bears whorls of green leaves/appendages (sepals), colored leaves (petals), male appendages (stamens), female appendages (carpels). The flower is generally defined as a highly specialized reproductive shoot, comparable to a normal shoot bearing leaves. The sepals and petals may be regarded as modified leaves. Stamens and carpels also show some resemblances to leaves in position, arrangement, internal structure and development. Based on these similarities leaves are comparable with leaves which bear reproductive organs. The terms microsporophyll and megasporophyll are often applied to stamen and carpel respectively. The flower thus, can be regarded as a group of sporophylls usually surrounded by sepals and petals.

In angiosperms (flowering plants), there are several modes of reproduction. Generally they can be arranged into two types, i.e., (i) asexual or vegetative and (ii) sexual types.

In asexual or vegetative reproduction, the offsprings are produced from the somatic cells, while in sexual reproduction there is fusion of male and female gametes.

1.3 THE FLOWER: A MODIFIED SHOOT

Flower is the reproductive part of the plant in which fruits and seeds develop. From morphological point of view flower is **a highly condensed and modified shoot** meant for reproduction.

In a typical flower there are four types of floral leaves. These are arranged in four whorls. Floral leaves are arranged on the swollen upper parts of flower stalk. The flower stalk is called **pedicel** and the swollen upper part is called **Thalamus (=torus).** In the thalamus are present nodes and highly reduced internodes. Floral leaves are borne on these nodes in whorls. The four floral whorls taken from periphery to centre are as follows:

1. Calyx: It is the outermost whorl and floral leaves of this whorl are called **sepals.** Their function is that of protection of inner whorls and also that of photosynthesis when green.

2. Corolla: It is the second whorl of floral leaves which are called petals. These protect the inner two whorls and also function for attraction of insects for pollination.
Calyx and corolla are called **accessory whorls** of the flower. In some plants although two whorls are present but they are not differentiated from one another. In such a case both whorls are called **perianth** and each part of the perianth is called **tepal.**

3. Androecium: This is third whorl of floral leaves and is the male reproductive organ of the flower. Each modified floral leaf is called **microsporophyll or stamen.**

4. Gynoecium (=Pistil): This is the last (fourth) central whorl of floral leaves and is the female reproductive organ of the flower. Each modified floral leaf is called **megasporophyll or carpel.** Gynoecium is made up of one or more megasporophylls (carpels). Androecium and gynoecium are called **essential whorls** of the flower.

In most of the flowers, the thalamus is condensed but in some, one or more internodes become long. If the internode between Calyx and Corolla becomes elongated it is called **anthophore** e.g., *Dianthus.* If the internode between corolla and androecium becomes elongated, it is called **androphore or gonophores** e.g., *Passiflora.* If the internode between androecium and gynoecium is elongated it is called **gynophores** e.g., *Capparis.* In *Gynandropsis pentaphylla* both androphore and gynophore are present and these taken together are called **gynandrophore or androgynophore.** In *Coriandrum and Foeniculum* the thalamus in between the two carpels gets elongated and after bifurcation protrudes out of the two carpels. It is called **carpophore.**

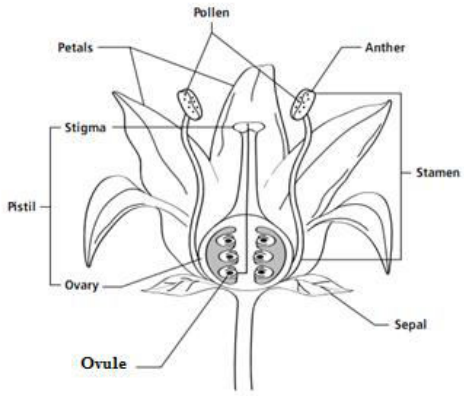

Fig.1.1: L.S. of a typical Flower

1.3.1-PARTS OF FLOWER

Bracts

Bracts are specialized leaves from the axil of which flowers arise. If a small leaf like structure is present on the pedicel of flower in between the bract and calyx of flower, it is called **bracteole.** The bracts may be of the below given types.

- **Foliaceous (Leafy) bracts:** They are leaf-like in appearance e.g. *Adhatoda, Gynandropsis.*

- **Petaloid bracts:** These bracts look like petals (are brightly coloured) e.g. *Bougainvillea* bracts look like petals of the flower while actual flower is tube like. Poinsettia (*Euphorbia pulcherrima).*
- **Epicalyx:** There is one or more whorls of bracteoles found at the base of calyx found in most members of Malvaceae e.g., **China rose.**
- **Involucre:** These are green coloured and in one or more whorls round and below the entire inflorescence e.g., Sunflower, Coriander.
- **Scaly bract:** At the base of each floret of members of Compositae e.g. Sunflower there is usually a membranous small bract different from involucral bracts.
- **Spathy bract:** This is large, boat-shaped bract enclosing a cluster of flowers or an inflorescence (e.g., Spadix) as in Banana, Maize, Palms.
- **Glumes:** These are small dry, scaly-bracts found in spikelet of Poaceae (Wheat). These are empty glumes, flowering glume (lemma) and palea.

Calyx

Calyx is the outer-most whorl and is made up of sepals which are usually green but sometimes coloured (petaloid) as in garden nasturtium. In dicotyledons usually the number of sepals is five and in monocotyledons usually three.

All the sepals of a whorl may be free **(polysepalous condition)** or they may be fused (**gamosepalous condition).** The sepals are superior in epigynous flower or inferior in hypogynous flowers. On the basis of time for which sepals remain attached upon the flower they may be **caducous** (if they fall just at the time of opening of flower from bud as in (poppy), **deciduous** (if they are attached on the flower until the flower weathers) e.g., mustard or **persistant** (remains attached to fruit e.g., tomato, brinjal).

Sometimes sepals are modified as follows:

- **Pappus:** In some plants e.g. Sunflower the sepals are modified into hairy structures called pappus and help in dispersal of fruits.
- **Leafy:** In *Mussaenda,* one of the sepals is modified into a large leaf like coloured structure.
- **Spinous:** In *Trapa* the calyx is persistent and modified into two spines.
- **Spurred:** In Larkspur, from the base of one of the sepals arises a tubular outgrowth called spur.
- **Hooded:** In *Aconitum* one of the sepals is modified into a hood thus covering the whole flower.

Corolla

It is the second whorl of flower and made up of petals. In dicotylendons the number of petals is usually five (variation from 2-10) and in monocotyledons three. The petals may be polypetalous or gamopetalous. The inferior and superior position of petals and their duration is like that of sepals. Petals are usually variously coloured. Sometimes they are green e.g., in *Artobotrys and Polyalthea.* Forms of corolla may be as follows:

I. Symmetrical and Polypetalous

- **Cruciform:** It consist of 4 petals arranged crosswise, each petal is cleaved e.g., Mustard.
- **Caryophyllaceous:** It consists of 5 petals, each with a comparatively long claw and the limbs of petals are placed at right angles to the claw, e.g., *Dianthus.*
- **Rosaceous:** It consists of 5 petals. Petals are with short claws or none. The limb spreads regularly outwards e.g., Rose.
- **Companulate or Bell shaped:** In this case the shape of the corolla is like bell e.g., *Physalis*.

II. Symmetrical and gamopetalous

- **Tubular:** In this case the corolla is tubular or cylindrical e.g. Central florets of Sunflower.
- **Infundibuliform or Funnel Shaped:** When the corolla is like a funnel e.g. *Datura.*
- **Rotate or Wheel shaped:** When the tube of the corolla is narrow and short and the limbs are at right angles to the tube e.g., Brinjal.
- **Salver shaped or Hypocrateriform :** When in a rotate type, the corolla tube is comparatively long e.g., *Ixora*

III. Monosymmetrical and polypetalous

- **Papilionaceous :** It is composed of five petals out of which posterior one is largest and known as **standard or vexillum.,** the two lateral ones, partially covered by standard are known as **wing** or alae, and the two innermost anterior smaller ones and united to form a boat shaped structure known as **keel or carina**. e.g., pea, gram (members of Fabaceae).

IV. Monosymmetrical and Gamopetalous

- **Bilabiate** (Two lipped): The whole corolla is divided into two lips; the upper lip and lower lip with the mouth gaping wide open. E.g., *Ocimum, Adhatoda.*
- **Ligulate or Strap-shaped:** When the upper part of corolla is flattened like a strap with the lower part forming a short tube e.g., outer florets of Sunflower.
- **Personate or Masked :** In this case also the corolla is bilabiate but the lip are so near to each other as to close the mouth of the corolla e.g., *Antirrhinun.*

Appendages of corolla

Sometimes the corolla or perianth has outgrowths of various types. It is saccate or gibbous in Snapdragon. In Balsam (*Impatiens),* Larkspur (*Delphinium)* the parianth is prolonged into a tube known as spur (spur contains nectar). Sometime by transverse splitting of corolla lobes, hairs, scales (free or united) may be produced known as **corna,** e.g., *Passiflora,*

Aestivation

The mode of arrangement of petals (also sepals) in a flower bud with respect to members of the same whorl is known as aestivation. It may be of the following types:

- **Valvate :** When the petals of a whorl lie close to each other i.e., neither overlap the margin of the adjacent petal nor are overlapped by the margin of the adjacent petal e.g. Mustard.

- **Twisted or contorted:** When one margin of petal of a whorl covers the margin of the adjacent petal and the other margin is covered over by the margin of adjacent petal. This order may be clockwise or anticlockwise e.g. China rose.
- **Imbricate :** When both margins of one of the petals are covered by the others and both margins of another one are external i.e. covering others and of the remaining three petals one margin is overlapped and the other overlapping e.g., *Cassia, Caesalpinia.*
- **Quincuncial:** It is a special type of imbricate aestivation in which 2 petals are external (both margins overlapping), 2 are internal (both margins overlapped) and in one petal one margin is overlapped and other overlapping e.g. *Calyx of Cucurbita maxima, Ranunculus.*
- **Vexillary:** Papilionaceous corolla e.g., beans, sweet peas.

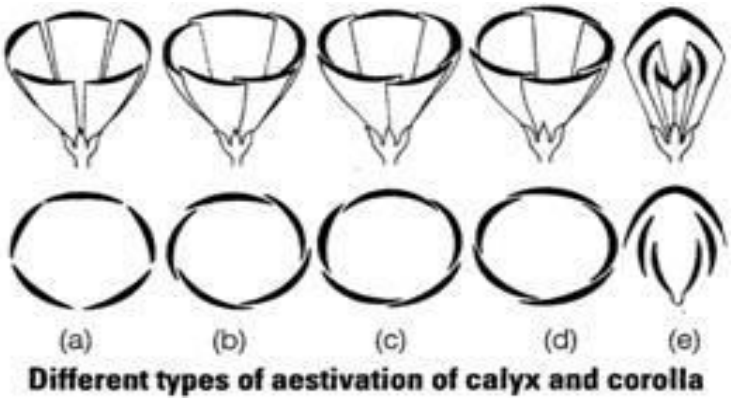

Fig.1.2: Types of Aestivation

Androecium

Andoecium is the third and male whorl of the flower and is made up of one or more stamens or microsporophylls. Each stamen has following parts:

- **Filament:** Lower thread like part of stamen is called filament. It may be absent in some stamens.
- **Anther:** This is the upper swollen part of stamen usually having two lobes (or theca) (in Malvaceae family it has only one lobe or theca). Each lobe contains 2 microsporangia. The two lobed anther is called **dithecous** anther. Anther with one lobe is called **monothecous.** The theca is filled with pollen grains (microspores). That part of anther to which connective is attached is considered as the back side of anther and the other side called face usually have longitudinal slits for dispersal of pollen grains. When the face of anther is towards centre of flower it is called **introse** e.g., tomato, when it is towards the periphery it is called **extrose** e.g., *Ranunculus.*

When the stamen does not have anther, the stamen is called **staminode.**

Attachment of filament to the anther

Anther can be attached to the filament in one of the following ways:

- **Adnate:** The filament runs along the back of the anther *Michelia* (Champa).

- **Basifixed: (**Innate) anther is fixed to the filament by its base e.g., *Datura.*
- **Dorsifixed:** The anther is fixed to the filament by its back and anther immobile e.g., Passion flower.
- **Versatile:** The anther is attached to the filament as in dorsifixed but is able to swing freely e.g., Wheat and grasses.

Cohesion of stamens

Stamens of an androecium may be free from one another and this condition is called **Polyandrous condition** but in many cases the stamens may be united to one another in different degrees e.g., when stamens are united by their filaments only (anther remaining free) it is called **adelphous condition.** When the stamens are united by their anthers only (filaments remaining free) it is called **syngenesious condition.** If however, filaments as well as anthers of different stamens are united it is called **synandrous condition.**

According to above given terms the cohesion may be of the following types:

- **Monadelphous stamens:** When all the filaments are united into a single bundle but anthers are free e.g., China rose, Lady's finger (Bhindi).
- **Diadelphous stamens**: When the filaments are united in two bundles the anthers remaining free e.g., pea, bean, gram.
- **Polyadelphous stamens:** When the filaments are united into more than two bundles but anthers are free e.g., Castor (*Ricinus*), Lemon (*Citrus*).
- **Syngenesious stamens**: When the anthers are united into a bundle but the filaments are free e.g., Sunflower.
- **Synandrous stamens**: When anthers as well as filaments of stamens are united throughout their whole length e.g., *Colocasia* and most cucurbits.

Adhesion of stamens

In adhesion, the stamens are attached to the tepals, petals or gynoecium. (1) It is **epiphyllous,** when stamens are attached to perianth e.g., in Liliaceae. (2) It is **epipetalous,** When they are attached to petals e.g., *Datura,* Sunflower. (3) It is gynandrous, when stamens are attached to gynoecium (either wholly or by anthers only e.g., *Calotropis* (Madar).

Length of stamens

Ocimum (lamiaceae) has four stamens, two of which are long and two short. This condition is called **didynamous** condition. In Brassicaceae (Cruciferae) family e.g., mustard, radish etc., of the six stamens four in inner whorls are longer and two in outer whorl are shorter. Such a condition is called **tetradynamous condition.**

Sometimes stamens may form hair like or scale like structure. Such outgrowth are called **staminal corona,** e.g., *Nerium.*

Gynoecium (Pistil)

Gynoecium is made up of one or more carpels (megasporophylls). Each carpel is differentiated into three parts

(a) the lower swollen part containing ovules; the **ovary,**

(b) elongated thread like structure attached to the apex of the ovary; **the style,**
(c) uppermost part, the **stigma.**

The gynoecium may be **monocarpellary** (made up of one carpel only) or **multicarpellary** (made up of many carpels). When the number of carpels in a gynoecium are two or more, they may be **free** or **united.** If they are free it is called **apocarpous gynoecium,** if they are fused it is called **syncarpous gynoecium.** Syncarpous gynoecium may be bicarpellary, tricarpellary, tetracarpellary, pentacarpellary or multicarpellary (if number of carpels exceeds five).

Sometimes the ovary has one chamber (locule), such ovary is called unilocular ovary but sometimes the ovary may be divided into several chambers e.g., may be bilocular, trilocular, tetralocular, pentalocular or multilocular depending upon the number of locules in the ovary.

Placentation

The ovules are attached on the inner surface of the ovary walls on one or more cushions called placenta. The manner in which placenta (placenatae) are arranged on the ovary wall is known as placentation**.**

Type of placentation

- **Marginal:** In the monocarpellary (Pea) or each carpel of multicarpellary apocarporous *(Ranunculus)* gynoecium, there is single placenta which develops along the junction of two fused margins.
- **Parietal:** When the gynoecium is formed by the fusion of two or more carpels by their adjacent margins, the ovary is unilocular and has two or more longitudinal placenta e.g., *Cucurbita,* Mustard, *Argemone.*
- **Axile:** In a multicarpellary syncarpous gynoecium the fusing margins grow inwards to meet in the center of the ovary to form an axis thus making the ovary multichambered (multilocular). The ovules are borne on the central axis e.g., *Solanum,* China rose.
- **Free central**: The ovary is unilocular and the ovules are borne on the axis in the center of the ovary e.g., *Dianthus.*
- **Basal**: The ovary is unilocular and a single ovule is borne at the base of ovary e.g., Sunflower.
- **Superficial**: The gynoecium is multicarpellary, syncarpous and large number of ovules are borne on the walls of loculi without specific order e.g., *Nymphaea* (Waterlily).

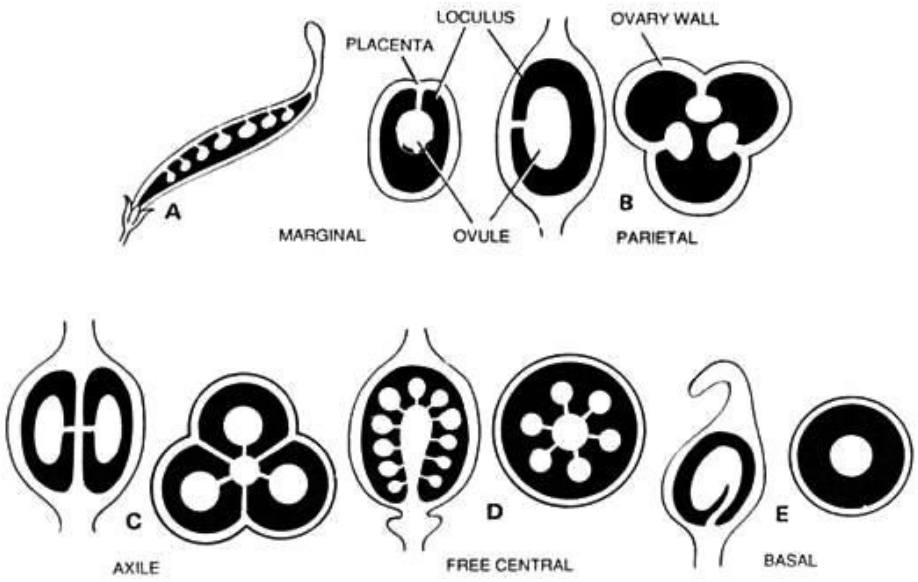

Fig.1.3: Types of Placentation

1.3.2-TYPES OF FLOWERS

In most flowers stamens and carpels are found in the same flower, such flowers are called **hermaphrodite or bisexual flowers** e.g., China rose, pea, cotton. In other flowers only one of the essential whorls is present. Such flowers are called **Unisexual flowers** e.g., Cucurbits, Mulberry etc. Flowers which have only stamens are called staminate flowers and those in which only carpels are present as **pistillate flowers.**

In most flowers all the four whorls are found, such flowers are called **complete** e.g., Cotton, China rose. If from a flower out the four whorls any one whorls is absent it is called **incomplete** flower e.g., Cucurbits.

A. On the basis of symmetry

- **Actinomorphic** (Regular, Symmetrical): Actinomorphic flowers can be divided (passing through center) by any vertical plane into two equal and similar halves e.g. Mustard, *Catharanthus roseus,* Brinjal.

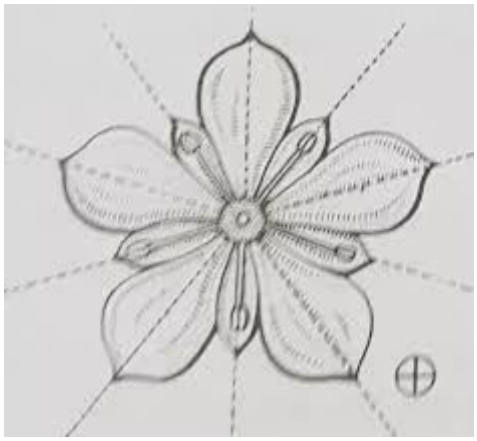

Fig.1.4: Actinomorphic Flower

- **Zygomorphic** (Monosymmetrical): Zygomorphic flowers can be divided into two equal halves by only one vertical division e.g., Pea, *Ocimum,* Larkspur.

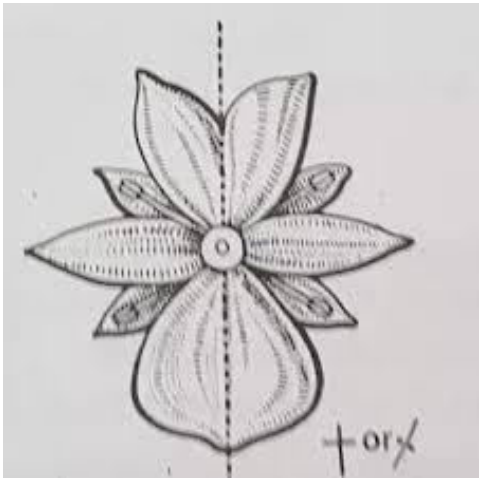

Fig.1.5: Zygomorphic Flower

- **Asymmetrical (irregular)**: Asymmetrical flowers cannot be divided into two equal halves by any vertical division e.g., *Canna.*

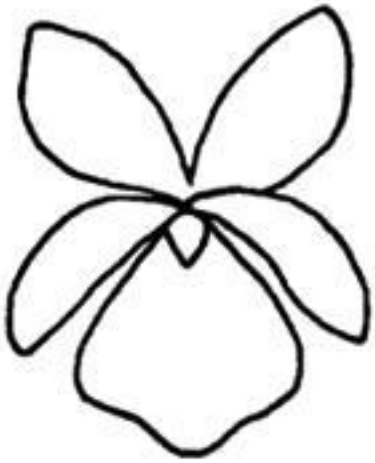

Fig.1.6: Asymmetrical Flower

Asymmetrical flower many be **isomerous or heteromerous.** A flower is isomerous when its whorls have equal number of parts or number in one whorl is multiple of the number of the other whorl. Isomerous flower may be bimerous, trimerous, tetramerous or pentamerous according to the number of parts in each whorl i.e., 2, 3, 4 or 5 or any multiple of it (carpel number is ignored). Trimerous flower are usually found in monocotyledons and tetramerous or pentamerous in dicotyledons.

When number in all the whorls is neither the same nor any multiple the flower is said to be heteromerous.

B. On the basis of Position of floral leaves

Normally the calyx, corolla, androecium and gynoecium of a flower are inserted on the thalamus in the sequence given above. But in many flowers the relative positions of the first three whorls in respect to ovary become disturbed due to unusual growth of thalamus. With reference to relative position of these whorls three conditions are seen:

- **Hypogyny:** In a hypogynous flower the ovary occupies the highest position on the thalamus, while the stamens, petals and sepals are separately and successively inserted below the ovary. Thus the ovary is said to be superior and rest of the floral parts as inferior e.g., China rose.
- **Perigyny :** In this condition the margin of the thalamus grows upwards to form a cup shaped structure called calyx tube enclosing the ovary but remaining free from it, carrying with its sepals, petals and stamens. The ovary is said to be half inferior, e.g., Rose, *Prunus.*
- **Epigyny:** In this condition the margin of thalamus grows further upward completely enclosing the ovary and getting fused with it and bear the sepals, petals and stamens above the ovary. The ovary in such cases is said to be inferior and the rest of the floral members superior e.g., Sunflower, Gourds.

(A) (B) (C)

Fig.1.7: Types of Flower. (A)Hypogynous, (B) Perigynous, (C) Epigynous

C. On the basis of presence of Accessory whorls

If accessory whorls are absent in a flower it is called **achlamydous flower** (naked flower), if out of the two accessory whorls only one whorl is present it is called **monochlamydous flower** and if both whorls are present it is called **dichlamydous flower.**

In addition to above types, flowers may be classified as:

Chasmogamous: A flower with exposed anther and stigma is chasmogamous flower.

Cleistogamous: A flower that does not open (i.e. anther and stigma is not exposed) is cleistogamous flower.

1.4 REPRODUCTION

The biological process through which an organism gives rises to young ones (offsprings) is called reproduction. The offsprings grow, mature and produce new offsprings. Thus, reproduction helps in continuity of species.

Types of Reproduction

In some plants of angiosperms, certain special modes of reproduction are also found. Generally, there are two types of reproduction, i.e., (i) asexual or vegetative and (ii) sexual types.

In asexual or vegetative reproduction, the offsprings are produced from the somatic cells, while in sexual reproduction there is fusion of male and female gametes. Thus

asexual/vegetative reproduction does not involve any fusion between the cells while sexual reproduction always involves fusion between two gametes.
In case of vegetative reproduction, any part of the plant, i.e., stem, root or leaf, is capable of growing into a new plant. Sometimes, in certain plants, buds and bulbils are formed, which develop into new plants.

In sexual reproduction, the gametes from male and female reproductive parts of the flower fuse to produce a zygote which develops into an embryo. This embryo remains inside the seed. Seed upon sowing germinates to produce a new plant.

In some plants, certain special modes of reproduction are also found which are commonly known as parthenogenesis, sporophytic budding, polyembryony, apospory, and micro-propagation. The production of synthetic or artificial seeds is also possible nowadays through tissue culture. These special modes have been discussed in the chapters ahead.

1.4.1. Vegetative/ Asexual Reproduction

It is a process in which a part of the plant body gets detached and develops in to a new independent plant.

In lower plants it occurs through binary fission, budding, fragmentation, gemmae, resting buds, soredia (in lichens) etc.

In higher plants, any part of the body maybe capable of vegetative propagation. Many plants produce modified stems, roots, and leaves especially for natural vegetative propagation. At the same time, man too has developed various methods of artificial vegetative propagation for many useful plants.

Types of methods of vegetative propagation

(1) Natural vegetative propagation
(2) Artificial vegetative propagation

(1) Natural vegetative propagation

Different plant parts are variously modified for vegetative propagation. The common structures that take part in vegetative propagation are root, stem, leaves and buds.

(i) Vegetative Propagation by Roots: The ordinary roots in many plants, such as *Dalbergia sisso*, *Populus*, *Guava*, *Murraya*, *Albizia lebbek* develop adventitious buds which grow to form new plants. Root tubers with adventitious buds occur in sweet potato, Tapioca, Yam, Dahlia and Asparagus.

When placed in the soil, the buds present on the tuberous roots grow in to leafy shoots called slips. Slips develop adventitious roots at their base. Slips are detached and planted to form new plants.

(ii) Vegetative Propagation by Stems: This is one of the most common and prevalent methods of vegetative propagation. Different plant parts, such as bulbs, runners, rhizomes, corms, tubers, offsets etc. help the plant to multiply under favourable conditions.

(a) Bulb: It is a modified shoot that has a very short stem and apical and axillary buds. Some of these grow to form shoots, e.g., *Allium* (onion), *Allium sativum* (garlic) etc.

(b) Runners: These are creeping stems which produce roots at nodes. Runners break at places and each piece develops into an independent plant, e.g., *Cynodon* (doob grass), *Oxalis* etc.

(c) Rhizomes: These are underground, horizontally growing stems. They have prominent nodes, internodes and axillary buds. Aerial branches sprout from the axillary buds which get separated from the rhizome and form new plants, e.g., Zinger.

(d) Corm: It is, in fact, a condensed rhizome that grows in vertical direction. The axillary buds present in the axil of scale leaves, produce daughter corms which later on form new independent plants, e.g., *Crocus* (Saffron), *Colocasia* (Taro), *Arisaema* etc.

(e) Tuber: It is a modification of underground stem. The 'eyes' or buds present on the tuber form new independent plants. Potato (*Solanum tuberosum*) is the most common example.

(J) Offset: It looks like a modification of runner, in being more or less thickened, prostrate branch with a tuft of leaves at the apex, e.g. *Pistia* (water lettuce), *Eichornia* (water hyacinth). They develop from the base of an old shoot or crown and after growing horizontally for some distance give rise to new crowns. They may break and form many independent plants.

(g) Aerial Shoots: A stem segment of *Opuntia* and other cacti develops into a new plant after falling on the soil. A similar segment of sugarcane with at least one node is used in agriculture to produce new plant.

(iii) Vegetative Propagation by Leaves: Leaves of a number of plants possess adventitious buds for vegetative propagation, e.g., leaf tips of walking fern (*Adiantum caudatum*), marginal notches in *Kalanchoe* and *Bryophyllum*.

In *Bryophyllum*, the marginal buds sprout while the leaf is attached to plant. In some other plants, the buds develop only when the leaf is injured or detached and fall on the moist soil, e.g., *Begonia*, and *Streptocarpus* sect. *Saintpaulia*,

(iv)Vegetative Propagation by Bulbils: They are fleshy buds which develop into new plants after falling on the soil, e.g. *Agave*, *Oxalis*, Pineapple (*Ananas camosus*),

Dioscorea etc. Some of them are modified floral buds, e.g., *Agave*. In *Dioscorea*, they develop in the axils of leaves while in *Oxalis* they occur above the base of fleshy root.

(2) Artificial vegetative propagation

In some plants, where vegetative reproduction by natural means is difficult to occur, special techniques can be used.

All the techniques or methods which are carried out by human beings to produce plants vegetatively are called artificial methods, These include stem cuttings, layering, root cuttings, grafting, gootee & micro-propagation (by tissue culture method).

1. Cutting: It is a simple method.

In this method, a suitable part of stem or root having node (about 20 to 30 cm long) is cut and it is planted in the soil, along with some nutrients. This cut part soon develops new roots and develops into a new plant.

Root cutting is commonly used in plants like lemon, tamarind, blackberry and raspberry etc. Stem cuttings are very common in plants like Rose, Croton, Sugarcane, Tapioca, China rose, Bougainvillea, Lemon, Coffee and Grape etc.

Leaf cutting also used to produce new offspring, in plants like *Sansevieria.*

2. Layering: It is one of the most common methods of artificial vegetative reproduction in plants.

In this method, a twig (branch) of a plant is bent down, below the level of soil. This bent part is called layer. A small incision is made in this layer (bent portion). Now the portion is covered with soil. Moisture is given at regular intervals. Soon this covered portion develops new roots and become separated (or can be cut) from main body, giving rise to new plant. This plant then can be shifted to some new location.

Layering is common in plants like-Jasmine, Strawberry, Grapevine and Cherry etc.

Types of Layering

(a) Tip layering: In tip layering, the tip of the current season's shoot is bent into the soil by digging a sloping hole. Soon the bent part develops roots. The tip also comes out as vertical shoot. The rooted shoot is separated, e.g., Blackberry, Raspberry.

(b) Serpentine layering: In serpentine layering, the basal branch is pegged down in the soil at several places to form a number of new plants from a single branch, e.g., *Clematis*.

(c) Mound layering: In mound layering, the basal part of a lower branch is bent down and covered with soil. The branch tip is kept outside the soil, e.g., Gooseberry.

(d) Trench Layering: Trench layering consists of pegging a branch or young plant (e.g., Walnut) in horizontal position in a trench. The horizontal shoot begins to develop vertical shoots. As soon as the shoots come out, their bases are covered to hasten rooting.

3. Grafting: It is the technique of joining parts of two different plants to from a composite plant. It can be done efficiently in those plants, which are closely related and have vascular cambium.

One plant, which has a strong root system, is selected as stock or stump (basal part). The branch of other plant (which is to be grafted) is selected as scion. Scion is usually selected from plants which have desired and superior characters.

The shoot of the stock is cut 20-30 cm above the ground. Leaves and buds are removed from this part. Now, complementary cuts are made in stock and scion, so that scion can be fitted exactly in the grooves of stock. After this fitting, the area is tied tightly with the help of a tough thread and then it is covered with grafting wax to avoid any infection. Grafting is carried out commonly in plants like Mango, Guava, Apple, Rubber plant, Citrus and Pear etc.

Types of grafting (On the basis of method of uniting two parts)

(a)Tongue grafting: In this case the stock and scion have almost same diameter. They are given oblique or sloping cuts. A small notch is given to ensure perfect fixing of scion into stock groove.

(b) Wedge grafting: In this case also, the stock and scion have same diameter. But a 'V shaped notch is given the stock while scion is cut like a wedge.

(c) Crown grafting: In this case stock has a larger diameter than scion. Many scions are selected and all of them are grafted on a single stock.

(d) Side grafting: In this case, lateral or side cuts are made in stock. One scion is fitted in each lateral cut of stock.

4. Gootee: It is also called air layering. It is commonly employed for the propagation of litchi, lemon, guava and orange etc.

In this method a healthy, leaf bearing branch of the main plant is selected. A ring of bark is removed (for a distance of 2-5 cm) from the basal part of this branch. The open part is covered with moist grafting clay (2 parts clay, 1 part cow dung, some fine cut way, moss or cotton and water). The graft is enriched with a root-promoting chemical. This area is then wrapped with a polythene paper to prevent desiccation and infection. This area develops small roots within 1-3 months. This branch is cut down and planted to a new location.

Advantages of Vegetative reproduction:

- It is easiest method of reproduction in plants.
- It helps to preserve the useful characters of the parental plant.
- It is a quick method of multiplication.
- It is a very helpful method of reproduction in those plants, which are sexually weak or have long dormant period of seeds or seedless.

- It helps in cloning and micro propagation of plants which in turn helps in developing a uniform population of plants.
- It helps to remove common infections through pruning, micro-grafting, and micro-propagation.
- Asexual methods like grafting helps in getting economically important plants, which have useful character of two different individuals.

Disadvantages of Vegetative Reproduction:

- Good qualities cannot be introduced nor bad characters eliminated in plants multiplied through vegetative propagation.
- Disease contacted by a parent spreads to all the daughter plants.
- Vegetative organs useful for propagation cannot be preserved for long.
- Vegetatively propagated plants are not as efficiently protected as the seeds are. They get easily decayed and are prone to various viral, fungal and bacterial diseases
- The plants may show degeneration due to absence of sexual stimulus and variation.
- Variability is absent. So, adaptability to changed environment decreases.

5. Micropropagation: The production of a large number of individuals from a small piece of plant tissue cultured in a nutrient medium.

Tissue culture: The aseptic culture of plant protoplasts, cells, tissues or organs under conditions which lead to cell multiplication or regeneration of organs or whole plants. Salient features of tissue culture are being given here as the detail discussion has been given in the Unit-9.

Steps of micropropagation

- Selection and maintenance of stock plants for culture initiation
- **Explant isolation** – Virtually any part of the plant can be used as explant like vegetative parts (shoot tip, meristem, leaves, stems, roots) or reproductive parts (Anthers, pollen, ovules, embryo, seed, spores). Shoot tip and auxiliary buds are most often used due to their meristematic nature and virus free region.
- **Surface sterilization** – Explants are surface sterilized by treating them with disinfectant solution of suitable concentration for a specific period. Ethyl alcohol, bromine water, mercuric chloride, silver nitrate, sodium hypochlorite, calcium hypochlorite etc. can be used as disinfectant.
- **Washing** – Wash with water.
- **Establishment of explant on appropriate medium** – There is no universal culture medium; however modifications of Murashige and Skoog basal medium (Murashige and Skoog, 1962) are most frequently used.
- Multiplication of shoots or somatic embryo formation (rapid) using a defined culture medium
- Rooting of regenerated shoots or germination of somatic embryos *in vitro*.
- **Hardening:** Transfer of plantlets to sterilized soil for hardening under greenhouse environment.

Advantages of Propagation by Tissue Culture Technique

- This technique enabled us to produce large number of plants in relatively short time.
- Disease free plants can be obtained by culturing the shoot apices (meristem) of infected plants.
- Very small sized explants can be used for micropropagation. This is impossible with conventional technique. It is important when limited explant is available.
- Material multiplied through micropropagation can be maintained in small place, packing and transport is also easy due to small size.
- Micropropagation is the only viable method of multiplying genetically modified cells or cells after protoplast fusion.
- In case of dioecious species, where one of the sex is more desirable then another, under such circumstances plants of desired sex can be selectively multiplied by this technique.
- The output is clean, healthy and pathogen free, as during micropropagation, fungi and bacteria are usually eliminated.
- Independent of the season hence can be carried out throughout the year.

Limitations:

- Requirement of sophisticated facilities.
- High production cost.
- Requirement of skill in handling and maintenance.
- Somaclonal variations may arise during *in vitro* culture when a callus phase is involved.
- For many valuable species suitable micropropagation techniques are not available so far (e.g. mango) but efforts are going on.

1.4.2 SEXUAL REPRODUCTION

The flower is a highly specialized reproductive shoot. Each typical flower consists of four distinct types of members arranged in four separate but closely set whorls, one above the other, on the top of a long or short stalk. The lower two whorls are called accessory whorls, and the upper two essential or reproductive whorls because only these two are directly concerned in reproduction.

The essential whorls consist of two kinds of sporophylls: microsporophylls or stamens and megasporophylls or carpels. Detailed structure, development and fate of stamens and carpels have been discussed in the following chapters.

1.5 SUMMARY

Flowers are the reproductive structures produced by plants which belong to the group known as Angiosperms, or 'Flowering plants'. This group includes an enormous variety of different plants ranging from buttercups and orchids to oak trees and grasses. There are about 250,000 known species. A flower is basically made up of four concentric rings of structures. There is an outer ring of modified leaves called sepals. These provide

protection to the flower before it opens and are usually green. This outer ring is known as the calyx. Inside the sepals is another ring of modified leaves called petals which are often brightly coloured. This layer is known as the corolla. Within the corolla are one or more stamens, which are the male reproductive structures. At the centre of the flower are the female reproductive organs (Carpels). The carpel consists of style, stigma and an ovary, which contains one or more ovules. There are many variations in this basic structural theme. In angiosperms there are several modes of reproduction. Generally, they are (i) asexual or vegetative and (ii) sexual types. In asexual or vegetative reproduction, the offspring are produced from the somatic cells, while in sexual reproduction there is fusion of male and female gametes. In case of vegetative reproduction, different parts of the plant, i.e., stem, root or leaf are capable of growing into a new plant, in addition to its normal functions. Some times in certain plants, buds and bulbils develop which form new plants. In sexual reproduction, the gametes from male and female organs of the flower fuse to produce a zygote. In some plants, certain special modes of reproduction are found, which are commonly known as parthenogenesis, sporophytic budding, polyembryony, apospory, and micro- propagation. The production of synthetic or artificial seeds is also possible now through tissue culture.

1.6 GLOSSARY

Anther: The part of the stamen where pollens are produced.
Angiosperm: A flowering plant whose seed is enclosed in an ovary.
Asexual propagation: The duplication of a plant from a somatic cell, tissue, or organ of the plant.
Calyx: The sepals of a flower; which enclose the unopened flower bud.
Carpel: The female part of a flower consisting of the stigma, style and ovary.
Coleoptile: A plumule sheath found in monocot seeds.
Coleorrhiza: A sheath surrounding radicle found in monocot seeds.
Corolla: The combined term for floral petals.
Grafting: The joining of two separate structures, such as two stems, so that by tissue regeneration they form a union and grow as one plant.
Graft union: Place where the rootstock joins the scion or top part of a grafted tree or vine.
Hypophysis: Lower most cell of a suspensor.
Monocotyledon: A flowering plant with one cotyledon.
Ovary: The enlarged basal portion of the pistil where ovules are produced.
Peduncle: The stalk of a flower.
Perfect flower: A flower that has either a pistil (or pistils) and stamens.
Petal: The parts next to sepals of a flower that are often conspicuously colored.
Pistil: The female part of a flower consisting of ovary, style and stigma. The ovary often supports a long style, topped by a stigma.
Receptacle: The part of a flower stalk where the parts of the flower are attached.

Scion: The upper part of the union of a graft. The shoot portion of a rootstock-scion graft.
Sepal: The outer parts of the flower (often green and leaf-like) that enclose a developing flower bud.
Staminate flower: Flower in which only the stamens (male reproductive parts) are present.
Stigma: The receptive part of the pistil where pollen grains germinate.

1.7 SELF ASSESSMENT QUESTION

1.7.1 Multiple Choice Questions

1. Flower in angiosperms
(a) Is a modified reproductive shoot (b) Possesses different floral appendages at successive nodes
(c) Have floral appendages which are modified leaves (d) More than one option is correct

2. When only the filaments of stamens are united into more than two bundles, the condition is called
(a) Monoadelphous (b) Diadelphous
(c) Polyandrous (d) Polyadelphous

3. In which placentation type, the ovary is two to many chambered and the ovules arise from central axis?
(a) Axile (b) Marginal
(c) Parietal (d) Basal

4. The four whorls of a flower are arranged on the
(a) Thalamus (b) Petiole
(c) Corolla (d) Stamens

5. In ..... placentation, the placenta forms a ridge along the venter suture of the ovary.
(a) Axile (b) Basal
(c) Free central (d) Marginal

6. The ray florets of sunflower has
(a) Superior ovary (b) Half inferior ovary
(c) Half superior ovary (d) Inferior ovary

7. Inflorescence with thick, fleshy axis and large coloured bracts is

(a) Spathe (b) Spadix
(c) Spikelet (d) Hypanthodium

8. Bisexual, sessile and bracteate flowers develop acropetally in
(a) Raceme (b) Panicle
(c) Spike (d) Corymb

9. The inflorescence characterized by having dimorphic flower is
(a) Catkin (b) Umbel
(c) Corymb (d) Capitulum

10. The largest flower in the world is that of
(a) Lotus (b) *Rafflesia*
(c) Giant cactus (d) Sunflower

11. Which of the following is a polycarpic plant
(a) Bamboo (b) Mango
(c) Pear (d) Pea

12. When the gynoecium is present in the topmost position of thalamus, the flower is known as
(a) Inferior (b) Epigynous
(c) Perigynous (d) Hypogynous

13. Pappus is a modification of
(a) Corolla (b) Calyx
(c) Androecium (d) Gynoecium

14. Anthesis means
(a) The state of expansion of a flower from a bud (b) Emergence of anthers from a corolla tube
(c) Dehiscence of anther (d) Elongation of pollen tube on stigma

15. A man without any knowledge of Botany thinks that it is a flower but actually it is an inflorescence
(a) Pea (b) Sunflower
(c) Rose (d) China rose

16. The placentation in which the ovary is unilocular and the ovules are borne on the axis in the center of the ovary is
(a) Basal (b) Free central
(c) Axile (d) Marginal

17. When the anther is fixed to the filament by its back and anther is immobile, the condition is called
(a) Adnate (b) Basifixed
(c) Dorsifixed (d) Versatile

18. When one margin of a petal of a whorl covers the margin of the adjacent petal and the other margin is covered over by the margin of adjacent petal, the aestivation is
(a) Valvate (b) Twisted
(c) Imbricate (d) Quincuncial

19. When the filaments are united into more than two bundles but anthers are free, the cohesion condition of stamens is
(a) Monadelphous (b) Diadelphous
(c) Polyadelphous (d) Synandrous

20. Gynobasic style is found in family
(a) Malvaceae (b) Cucurbitaceae
(c) Lamiaceae (d) Solanaceae

1.7.1 Answers key:

1. (d), 2. (d), 3.(a), 4. (a), 5. (d), 6. (d), 7. (b), 8. (b), 9. (d), 10. (b), 11. (b), 12. (d), 13.(b) 14.(a), 15. (b), 16. (b), 17. (c), 18. (b), 19. (c), 20. (c)

1.7.2 Short answer Questions

1. Describe the reproductive parts of a flower.
2. Give examples in support of axis nature of thalamus.
3. Describe a hypogynous flower.
4. What is aestvation?
5. What is parietal placentation?
6. Mention one difference between chasmogamous and cleistogamous flower.
7. Define the term dithecous.
8. Differentiate between sexual reproduction and asexual reproduction.
9. Describe two forms each of symmetrical polypetalous and gamoptalous petals.
10. Differentiate between didynamousand tetradynamous condition of stamens giving examples.

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Important website and links

- http://biology.clc.uc.edu/courses/bio106(accessed on March, 2016).
- http://www.biologyreference.com/(accessed on March, 2016).
- www.BiologyDiscussion.com

1.10 TERMINAL QUESTIONS

1. Describe different types of aestivation.
2. Discuss the types of flower on the basis of (i) symmetry and (ii) position of floral leaves.

3. Describe natural vegetative propagation in flowering plants.
4. Discuss the salient features of tissue culture.
5. Describe in detail about the artificial modes of vegetative reproduction.

UNIT-2: DEVELOPMENT OF MALE GAMETOPHYTE

2.1 OBJECTIVES

Dear students in the previous unit you studied the floral details and different types of reproduction in the angiospermic plants. In this unit you will learn about the:

- Male reproductive part of the flower
- Morphology and Anatomy of the stamen
- Microsporogenesis
- Development of the male gametophyte
- Brief introduction about pollination

2.2 INTRODUCTION

The stamen also known as microsporophyll consists of a filament, anther and a connective. Filament is a thread like structure bearing anther at its tip. Anther consists of two anther lobes connected by a tissue known as connective. Each anther lobe contains two pollen sacs or microsporangia, present at the corners of the 4-lobed anther. Young anther is more or less oblong in shape in section and made up of homogeneous mass of meristematic cells while mature anther contains four pollen sacs (microsporangia) having many pollen grains. Pollen grain is the first cell of male gametophyte and two male gametes develop in each male gametophyte.

2.3 ANTHER:

2.3.1 Development of anther (microsporangium)

A typical anther is tetrasporangiate (having 4 microsporangia). Central tissue of anther is sterile and known as connective tissue. Anther lobes are on its lateral sides. Each anther lobe is having two microsporangia (dithecous). These microsporangia are separated by layer of sterile tissue. In some plants such as *Moringa* and *Wolffia* each anther lobe has only one microsporangium (monothecous).

T.S. of a young anther shows undifferentiated mass of cells surrounded by epidermis. Below the epidermis, at each corner, some cells become differentiated from others by their dense protoplasm known as archesporium or archesporial cells (Fig. 2.1 B). Each archesporial cell then divides mitotically and forms an outer primary parietal cell and an inner primary sporogenous cell.

The outer primary parietal cells form primary parietal cell layer at each corner (Fig.2.1.D. Below the parietal cell layer, the primary sporogenous cells remain in groups *i.e*. the

sporogenous tissue. The cells of primary parietal layer then divide both periclinally (along the periphery) and anticlinally (at right angle to the periphery) and form multilayered wall (Fig. 2.1 E).

The innermost layer of wall, which remains in close contact with the sporogenous tissue, functions as nutritive layer, called tapetum (Fig. 2.1 F). It provides nourishment to the developing pollen grains. The layer just below the epidermis is called endothecium. The sporogenous layer may function directly as pollen mother cell or it may divide and redivide and the last cell generation becomes pollen mother cells. Development of microsporangium thus takes place from a group of initial cells and this is called eusporangiate development.

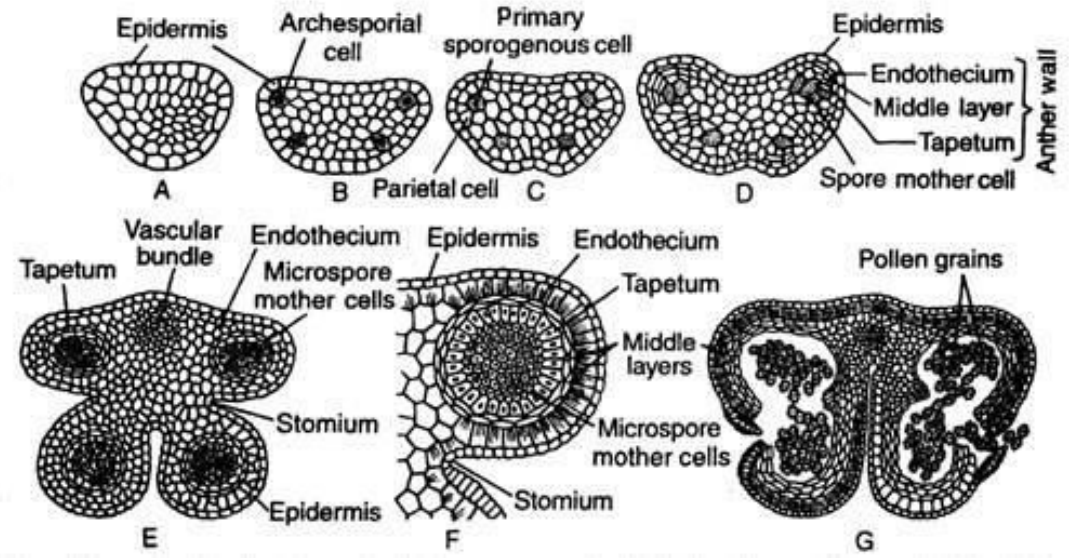


Fig. 2.1 Stages of the development of anther A to D. Differentation of archisporial initials. Differentiation of primary parietal cells and primary sporogenous cells E. T.S. Anther showing four sporangia containg microspore mother cell and micropore tetrads F. Enlarged microsporangia with all anther wall G. T.S. young anther showing dehiscence of pollen grains

The sequential detailed description and function of different wall layers (from outer to inner) are as follows:

2.3.1.1. Anther wall: The mature anther wall consists of epidermis, followed by an endothecium, middle layers (2-3 layers) and innermost tapetum.

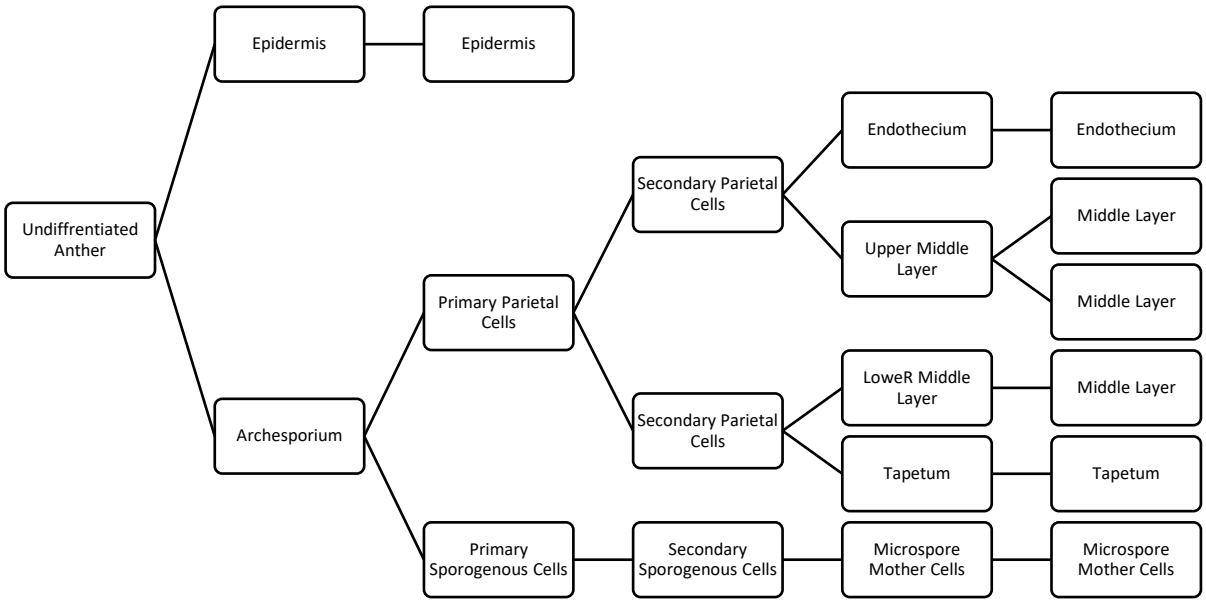


Fig 2.2 Schematic representation of formation of various anther wall layers and microspore mother cell

A. Epidermis: It is the outermost layer of a young anther. During development it undergoes various anticlinal divisions only. In mature anther it is much stretched and flattened. It is protective in function.

B. Endothecium: Generally it is single layered situated immediately below the epidermis. The endothecium originates from the parietal layer, this layer attains maximum development when the anther is ready to dehisce for discharge of pollens. The cells of this layer are radially elongated and develop fibrous thickenings (where there is longitudinal dehiscence). These cells are hygroscopic in nature and help in dehiscence.

C. Middle layers: Usually one to three middle layers are found below the endothecium. They become crushed at the time of meiotic division in the microspore mother cell. In some plants such as *Ranunculus* and *Lilium* it may be persistent.

D. Tapetum: This is the innermost parietal layer of anther wall below the middle layer. Usually single layered rich in reserve food material and passes the food to the sporogenous cells. Thus it serves as a nutritive layer for the developing microspores. Typically the tapetum is composed of single layer of cell characterized by dense protoplasm.

The cells of the tapetum show polyploidy due to endomitotic divisions. Tapetal cells absorb food from the middle layers and provide nutrition to the microspore mother cells. The cells of tapetum secrete hormones and enzymes. The tapetum layer disappears in the mature anther. In Some plants *e .g. Alectra thomsonii* and *Nigella demasecena*, tapetum

is **Dimorphic (P - tapetum and C - tapetum).** The tapetum may be glandular or amoeboid based on the behaviour of the cells during sporogenesis.

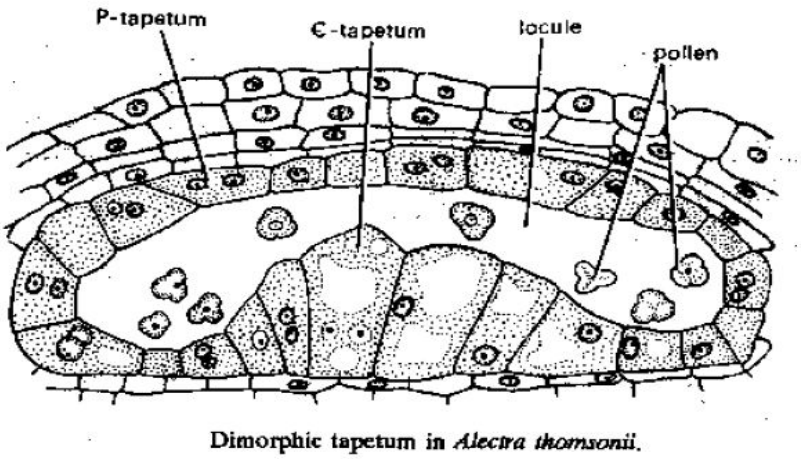


Fig. 2.3 Dimorphic tapetum

1. **Amoeboid tapetum:**

It is also called periplasmodial tapetum.. The inner and radial walls of the tapetum break down due to the action of hydrolytic enzymes and their protoplast penetrates between the pollen mother cells and developing pollen grains. Microspore mother cells are surrounded by the periplasmodium and receive nourishment from them. The bodies of protoplast are known as **periplasmodium.** Amoeboid tapetum is considered as the primitive type. It is found in primitive Angiosperms usually in hydrophytes. Eg., *Alisma, Tradescantia, Typha, Saggitaria, Potamogeton*.

Such type of tapetum absorbs food from the middle layers so the middle layers become dead. When the anther dries up, the tapetal periplasmodium gets dehydrated and coated over the surface of pollen grains, thereby helping in the formation of exine. This tapetal plasmodium remains associated with the pollen grains till their maturity after intrusion; they fuse with each other and form a mass of tapetal periplasmodium. Thus here tapetum is concerned with transportation as well as storage of food.

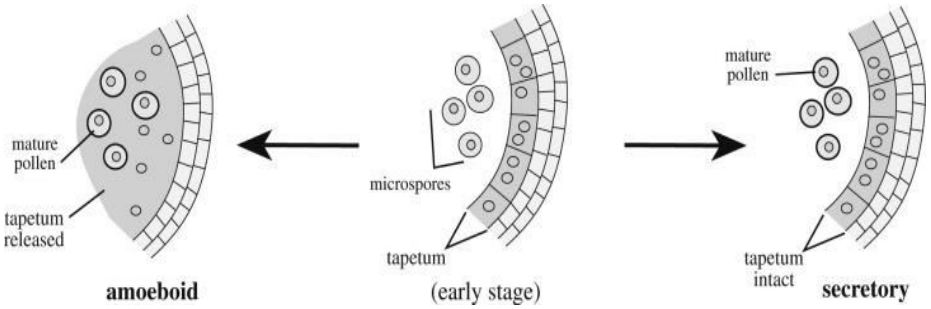


Fig. 2.4 Types of Tapetum

2. Glandular (or secretory) tapetum:

It is more common in angiosperms in which tapetum cells remain as such in their original position, throughout the microspore development. These cells secrete nutrient material which is absorbed by the developing spores. It is developed type of tapetum. It does not degenerate quickly. It absorbs nutrients from the middle layers and secrete into the cavity of the microsporangium At the initial stages of .the development, the cells of the glandular tapetum, contain small bodies, called pro-ubisch bodies before their degeneration, which are involved in the external thickening of the exine of the spore wall. It is also called parietal tapetum. Just before the pollen mother cells undergo meiosis, the walls of the tapetal cells become thick and there is a considerable increase in the number of ribosomes and pro-ubisch bodies with the completion of pollen development pro-ubish bodies pass into the anther locule from the tapetal cells and they are now called Ubisch bodies, which now get coated over the pollen grains. They secrete their substances from their inner faces. Secretary tapetal cells are thin and possess almost all cell organelles like mitochondria, plastids, dictyosomes etc. The cells of glandular tapetum remain intact throughout microspore development.

Function of tapetum: 1.The nutrients are transported through tapetum to the sporogenous tissue and protect pollen from ultra violet. 2. Tapetum plays an important role in the formation of exine. 3. Tapetum is involved in the synthesis of callose which release microspores in a tetrad by degrading callose wall **4.** Pollen kit (Lipids and carotenoids) is formed by tapetal layer act as insect attractant.

2.3.1.2. Sporogenous tissue

The primary sporogenous cells either directly function as spore mother cells or divide mitotically into a number of cells which function as spore mother cells. Each microspore mother cell divides meiotically to produce four microspores or pollen grains arranged tetrahedrally (Fig. 2.6) which will have half (n) the number of chromosomes.

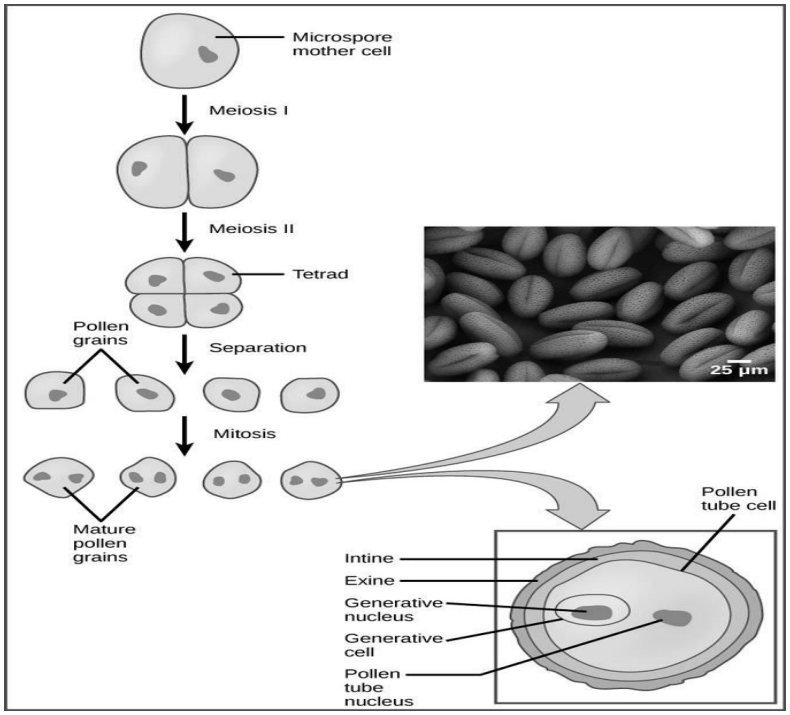


Fig. 2.5 Stages of microsporogenesis

The stimulus for inducing meiosis is not clear yet but it probably originates in some other part of the plant and transmitted to the anther specifically to the sporogenous tissue only. In the pre-leptotene stage the PMCs (pollen mother cells) in an anther locule are interconnected by plasmodesmata..At this stage plasmodesmatal connections also exist between the tapetal cells and PMCs. With the entry of PMCs into meiosis the connections between tapetal cells and the PMCs are broken and the walls of the PMCs become thicker by the deposition of callose (β-1,3 glucan)..

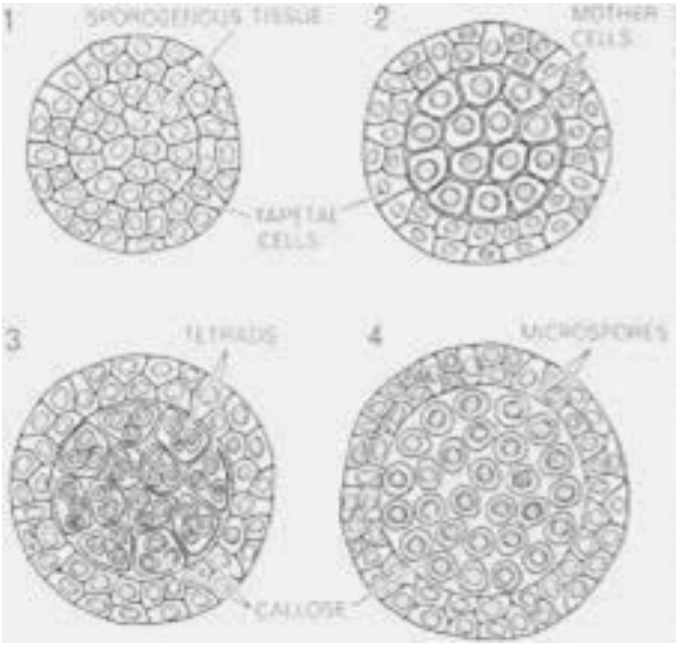


Fig. 2.6 Diagrams showing celluar interconnections in developing anther

The deposition of callose usually starts at the corners of the cells between the plasma membrane and the original wall. The latter is finally degraded. Concurrently with the deposition of callose, the plasmodesmatal connections between the PMCs are replaced by massive cytoplasmic channels. These channels are 1-2 μM in diameter. They attain their maximum development in the zygotene –pachytene stage. Thus, at this stage the whole mass of PMCs in an anther locule forms a large meiocytic synctium. In some families such as Mimosaceae and Orchidaceae, the sporogenous tissue in an anther lobe is partitioned by plates of somatic cell . In such plants the synctium is formed in small groups. The massive cytoplasmic channels provide passage for the movement of cytoplasmic contents from one cell to the other. Gates (1911) had noted the movement of nuclear material from one PMC to another and termed the phenomenon as **cytomixis.** The cytoplasmic continuities of the PMCs impose a mutual influence of one cell over the other. This helps in maintaining a close synchrony during meiosis in the large number of PMCs in an anther locule.

At the end of meiotic prophase the callose wall of PMCs close up and cytoplasmic channels are cut off. With this all the PMCs become independent and go through the rest of meiosis as isolated cells which contribute additional walls between the microspores of PMCs.

2.4 DEVELOPMENT OF MICROSPORES OR POLLEN GRAINS

2.4.1. Formation of microspores or pollen grains (Microsporogenesis):

Development of microspores from microsporocytes is termed as **microsporogenesis**..The sporogenous tissues, formed by archesporial cells divide many times to form pollen mother

cells (or microspore mother cells) these are diploid cells. Each such cell divides meiotically (by meiosis) and forms four haploid microspores or pollen grains. The division is of two types in various angiosperms – simultaneous type and successive type which are determined by the timing of cytokinesis, which is the formation of a plasma membrane and cell wall that divides one cell into two.

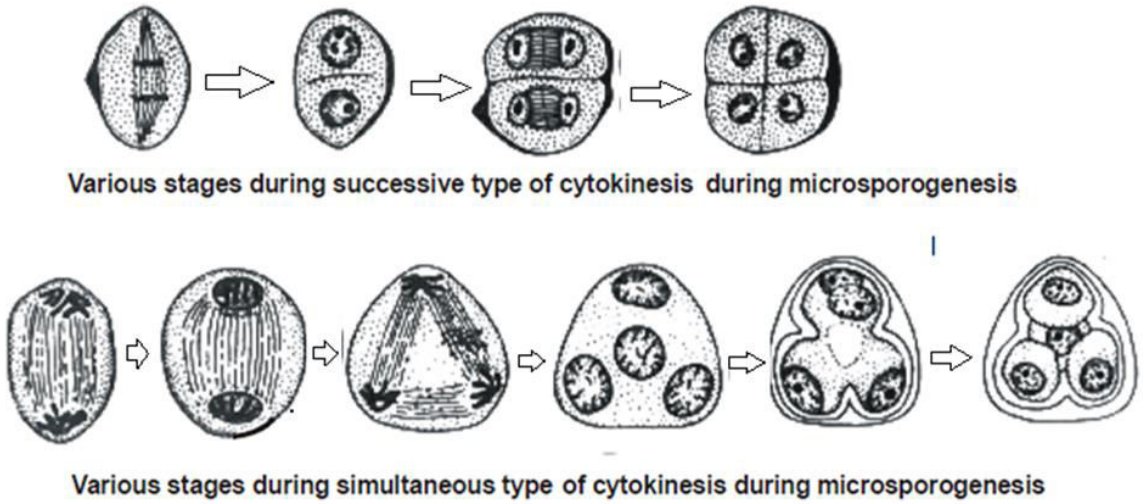


Fig. 2.7 Successive and simultaneous types of cytokinesis

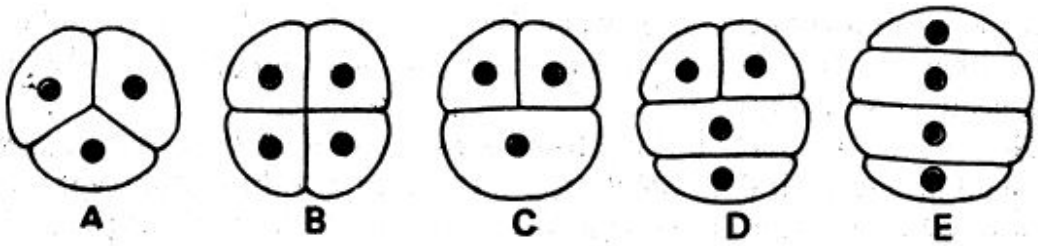
In simultaneous division meiosis I is not followed by cytokinesis. Cell wall formation takes place only after the completion of both Meiosis-I and Meiosis-ll. While in successive type of division, cytokinesis occurs after both phases of meiosis (I and II)

Microspore mother cells → Meiosis I → Meiosis II (pollen grain)
Diploid 2 haploid cell 4 Microspore

The four microspores initially remain associated with each other. This group of four haploid (n) pollen grains is called microspore tetrad. In *Zostera* some microspore mother cells become sterile and provide nutrition to rest of microspore mother cells.

Following types of tetrad arrangement is found in angiosperms

1. **Tetrahedral** - Four haploid microspores arranged in tetrahedral form *e.g. Capsella* - dicotyledons.
2. **Isobilateral** – In monocotyledons this tetrad occur.
3. **Decussate type** - Two microspores lies at the right angle of each other e.g. *Magnolia*
4. **T-Shaped tetrad**- Out of 4, two microspores lies longitudinally and two transversely to it e.g. *Aristolochia.*
5. **Linear tetrad** - Pollens arranged in linear manner in this tetrad *e.g.*, *Halophia.* In *Aristolochia elegans*, all the 5 types of microspore tetrads are found.



A-E. Various types of pollen tetrad : A Tetrahedral, B. Isobilateral, C. Decussate, D. T-shaped, E. Linear.

Fig. 2.8 Various Type of pollen tetrad

Unusal pollen tetrad

In the members of Asclepiadaceae (*Calotropis*) and Orchidaceae family, all the pollen grains joined together to form **Pollinium.** Pollinium of *Calotropis* is called **Translator apparatus (Fig 2.9 B).**

Polyspory is the occurrence of more than four pollen grains **e.g.** *Cuscuta***.**

When four pollens of tetrad join together permanently they are called **Compound pollen** e.g. *Drosera, Typha*. In Mimosaceae family 6-8 pollen grains grouped together to form special compound pollen called **Massullae** *e.g.* Mimosa.

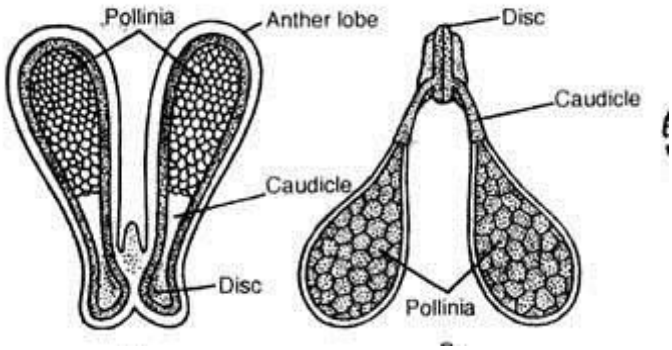


Fig.2.9 Pollinia of A. Orchis and B. Calotropis

Pollen grains of some plants cause allergy are called **aero allergens** *e.g. Chenopodium, Parthenium, Sorghum, and Amaranthus.* ("Hay fever" is caused by pollens of *Ambrosia*). Only one pollen grain is formed from pollen mother cell in Cyperaceae family *e.g. Cyperus.* Typical Female embryo sac like **Eight Nucleated embryo sac** type of pollen found in ***Hyacinthus***.

The enzyme callase disintegrates the callose (polysaccharide) present in pollen tetrad. As the anthers mature and dehydrate, the microspores dissociate from each other and develop into pollen grains. In each microsporangium, thousands of pollen grains are formed and released with the dehiscence of anther.

Structure of pollen grain (microspore):

Microspore (or pollen grain) is the first cell of the male gametophyte. It is unicellular, uni-nucleated, haploid and spherical structures, which develop to give rise to male gametophyte

These are of various shapes — polyhedral (*Sonchus palustris* of Asteraceae), cubical (*Basella alba* of Basellaceae), trigonal (common in Onagraceae), cylindrical (*Rheo discolor* of Commelinaceae) etc. The size of the pollen grains generally varies from 10-80µm, but the size may be even 100µm in diameter. The pollen grains have two walls outer exine and inner intine.

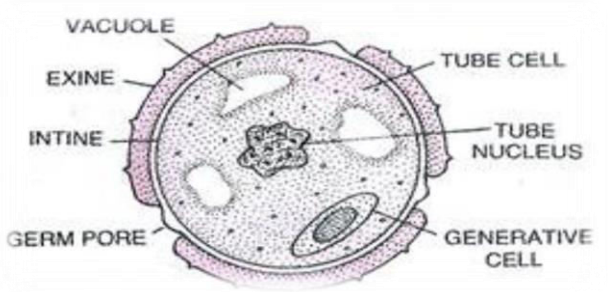


Fig 2.10 Cross-section of pollen grain

The exine is differentiated into of outer sexine and inner nexine, which is thick and cuticularised layer. Exine consists of eketxine (outer) and endexine (inner) Extectine further consists of a foot layer, baculate layer (middle) and an outermost tectum. Tectum contribute characteristic texture to exine.

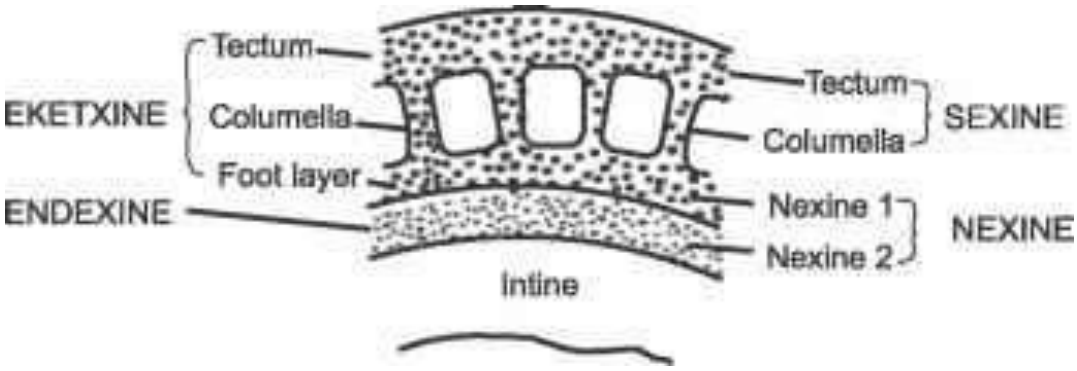


Fig 2.11.Detailed Structure of tectate pollen wall

The exine shows different types of outgrowths. It contains a resistant fatty substance, called **sporopollenin**. It is non- biodegradable. Fossils of pollen grains are found in good condition due to presence of this substance. Mineral resources like petroleum, coals etc.

in the earth can be predicted by the presence of pollen-grains in fossils. The internal layer is thin soft and delicate called intine. It is made up of pectin and cellulose. Usually, at some points sporopollennin is not deposited on exine. These thin points or places are called **germ pores**.

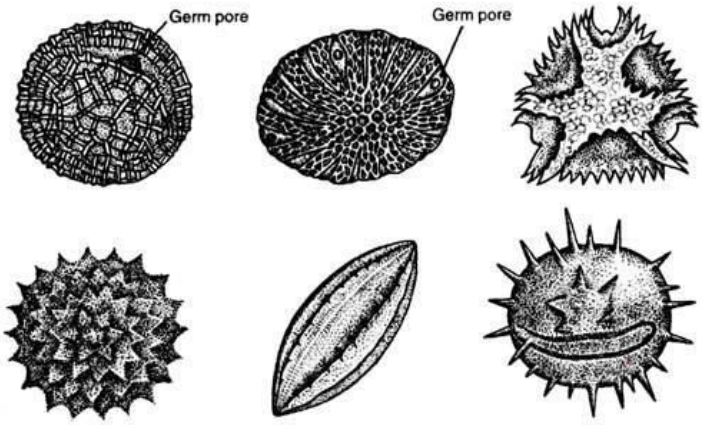


Fig 2.12 Different pollen grains showing various types of sculpturing

The morphology and texture of exine has taxonomic importance. The study of pollen and its exine structure is called Palynology. **Erdtman is considered as Father of Palynology** while **Father of Indian Palynology** is **P.K. Nair.** .

Presence of three germ pores (tricolpate) is generally in dicots. In monocots, germ pores are absent and there is one **germinal furrow.** Such plants in which pollination takes place by insects their pollen grains have oily layer around the pollen grain. It is called **pollen-kit**. This oily layer protects the pollen grains from the harmful ultraviolet rays and its sticky surface helps for attachment with insect's legs. Chemically the pollen grains are composed of carbohydrate (25-48%), protein (7-26%), water (7-16%), and Fats (1-15%). The germ pores are important because these mark the origin of pollen tube.

2.5 MICROGAMETOGENESIS

Microspore or pollen grain is the first cell of the male gametophyte, which contains only one haploid nucleus. During early stages of development, it remains within the microsporangium. The germination of pollen grain starts before the dehiscence of microsporangium.

Development of the male gametophyte has following steps –

(a) Development before pollination.

(b) Dehiscence of anther and dispersal of developing pollens.

(c) Pollination

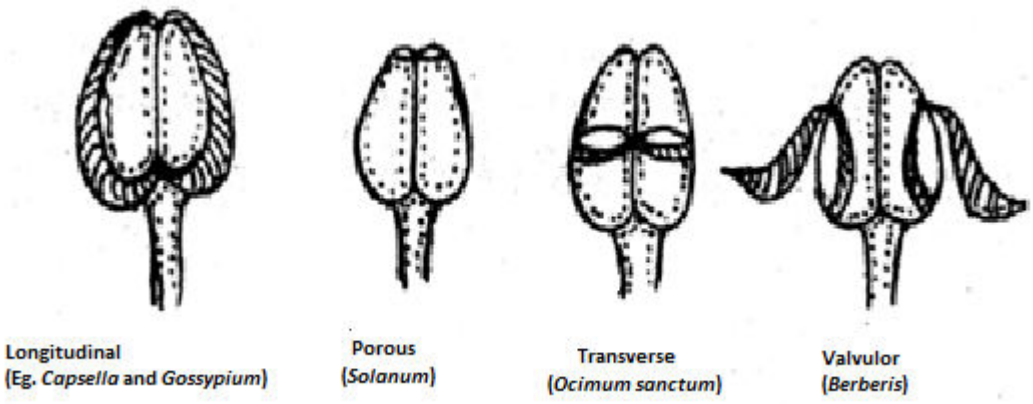
(d) Development after pollination

2.5.1. Development before pollination.

The single nucleus of the pollen grain divides mitotically to form two nuclei- tube nucleus and generative nucleus before the pollination.. Tube nucleus is smaller and placed mostly at the center of the cytoplasm while generative nucleus is comparatively larger lying near the cell wall. Nuclear division is followed by the development of cell wall between the two to form unequal cells, a smaller generative cell and larger tube or **vegetative cell**. The generative cell gets detached and lies freely in the vacuolated cytoplasm. Generative cell divides and become two celled or two nucleated. Pollen grains are shed for pollination at this stage. It means at the time of pollination two or three cells are present in the pollen grains... Further development of pollen grain occurs on stigma of gynoecium.

2.5.2. Dehiscence of anther and liberation of developing pollens.

The changes take place in during the maturation of pollen grains. The tapetal layer and middle layers are used up slowly in nourishing the developing pollen grains.



2.13 Different modes of anther dehiscence

The sterile tissue between the two microsporangia of one lobe disappears by dissolution of cells forming one large chamber having a common epidermis and endothecium. The loss of water in the cells of endothecium makes them dry. These cells start shrinking causing a tension in the endothecial layer which ultimately ruptures at thinnest point i.e. stomium. Rupturing takes place with a jerke and pollen grains are thrown away in the air as well as on the surface of anther.

Usually dehiscence of the microsporangia takes place in the form of long fissure at the place where both pollen sacs of a lobe meet together. This type of dehiscence is called longitudinal. Besides this dehiscence sometimes fissures may be in the form of valves (e.g. *Barberry*) or transverse or in the form of apical pores (e.g. *Solanum nigrum*).

2.5.3. Pollination:

Transfer of pollen grains from anther to the stigma of a pistil is called pollination. **It is of two main types:** (i) self-pollination (ii) Cross-pollination.

2.5.3.1. Self pollination:

Transfer of pollen from anther of a flower to the stigma of the same flower (or flower of same plant) is called self pollination. Self-pollination can be of two types, autogamy and geitonogamy,

(1) Autogamy:

This type of self-pollination occurs in bisexual flowers. Here the stigma of a flower is pollinated by its own pollens. Autogamy occurs by following three methods:

(a) Homogamy:

The anthers and stigmas of open flowers are brought together by growth, bending or folding *e.g. Catharanthus* (*Vinca*), *Mirabills* (four O'clock plant)

(b) Cleistogamy:

Such flowers remain closed during pollination .Thus; stigma receives only the pollen of its own flower. *e.g. Oxalis, Arachis hypogea, Commelina*,

(c) Bud Pollination:

The sex organs develop before the opening of bud, thus internal pollination takes place. *e.g*. Pea, Wheat, Rice, etc.

(2) Geitonogamy:

In this type of pollination, the pollen grains of one flower are transferred to the stigma of another flower in same plant or genetically similar plant.

2.5.3.2. Cross pollination/Allogamy:

Transfer of pollen grains from the anther of the flower of one plant to the stigma of the flower of other plant by the help of agents is known as cross pollination. Agent may be abiotic (non living) or biotic (living). It is commonly seen in dioecious plants. It is highly advantageous than self pollination due to formation of new genotypes.

Types of Cross Pollination:

Cross pollination by abiotic (non living) agents:

1. Pollination by Wind or air is known as Anemophily.

2. Pollination by Water is known as Hydrophily.

Cross pollination by biotic (living) agents.

1. Entomophily: Pollination by insect is known as Entomophily. *E.g*., *Salvia, Cestrumus.*

2. Ornithophily: It is the Pollination by birds *e.g*., *Bombax, Callistemon.*

3. Zoophily: It is pollination by animals.

4. Malacophily: Pollination by Snail is known as malacophily. *E.g., Lemna, Colocasia, Diptera.*

5. Chiroptrophily: Pollination by Bats is called chiropterophily *e.g., Kigelia, Anthocephalus, Adansonia, Bauhinia*

6. Mirmicophily: It is pollination by ants.

7. Ophiophily: Pollination by Snakes is known as ophiophily.

Adaptations for Cross- Pollination:

The cross-pollinated plants adapt several mechanisms for successful cross-pollination. Some of these adaptations are:

1. Uni-sexuality or Dicliny:

Plants bearing unisexual flowers are dioecious (*e.g. Cannabis, papaya*). Unisexuality is best adaptive character for cross pollination.

2. Self-sterility or incompatibility:

Plants such as *Passiflora,* Potato*, Malva,* and *Abutilon* show self-incompatibility. In these plants pollen grains from an individual flower cannot pollinate its own carpel as these pollen grains fail to germinate on stigma of the same flower. Self- sterility in plants is under genetic control which prevents the eggs in ovules being fertilized by pollens from the same plant.

3. Dichogamy:

It is a condition where in some hermaphrodite flowers stamens and carpels of a flower do not mature at the same time. It is of two types:

(i) Protandry: Here anthers mature much earlier than the carpels of a flower, *e.g.,* Sunflower, *Tagetes, Jasminum, Foeniculum etc.*

(ii) Protogyny: when carpels of flower mature much earlier than its anthers, e.g. Rose, *Ficus benghalensis,* etc.

4. Heterostyly:

It is the phenomenon of disparity in the length of style and stigma that's why effective self pollination is not possible, e.g., *Primula, Lathynis, Oxalis* etc.

5. Herkogamy:

Here, homogamous flowers develop some unusual structure to facilitate successful cross-pollination. For instance, in caryophyllaceous flowers, the stigma grows much beyond the limits of stamens so that its own pollen-grains fail to reach its own stigma. In *Calotropis*, the corolla acts as hood in between androecium and gynoecium.

Various agencies of cross pollination (Allogamy):

Most of the plants are chasmogamous type (i.e, they expose their anthers and stigma to the pollinating agencies). As the pollen grains do not have locomotory structures, they are transferred from anther to the stigma, with the help of certain agencies, called as pollinating agencies. Some of them are wind (air), water, insects, bats, birds and even by man.

1. Anemophily:

Anemophily refers to the pollination by wind (air). It is aprimitive type pollination. Following characters: are shown by wind pollinated flowers.

(a) Flowers are inconspicuous and not showy.

(b) They are devoid of fragrance and nectar.

(c) They produce a very large amount of pollen grains, as considerate amount of pollen never reaches the proper stigma.

(d) Anthers are versatile, swinging freely in air.

(e) Pollen grains are dry, light and smooth walled.

(f) Stigma is large, branched and bushy to catch pollens from air. *e.g.* family poaceae.

2. Hydrophily:

Pollination through water current is called hydrophily. It may take place completely under water (hypohydrophily) or may takes place on the water surface (epihydrophily). Hydrophilous plants like anemophilous flowers are characterised by floral envelop which are highly reduced or even absent. It is commonly found in plants like *Zostera, Ceratophyllum* and *Vallisneria.*

3. Entomophily:

Insects are the chief pollinators. Insects helping in pollination are bees, flies, beetles and moths. Some insects are diurnal (visiting flowers which opens in day time) *e.g*. bees, flies, beetles. While some are nocturnal (e.g., moth). They visit flowers which open after sunset.

Insects pollinated flowers show following characters:

(a) Plants pollinated by diurnal insects have brightly coloured flowers.

(b) Pollen grains and stigma are having a rough surface facilitating insect limbs to stick.

(c) Nectar (sugary fluid) glands are present in many insect pollinated plants which may be situated on thalamus, sepals, petals, carpels or base of ovary. Insects visit flowers for nectar.

(d) Flowers which open at night have white colour and fragrance.

(e) Special pollination mechanism is seen in some entomophilous flowers. In *Centaurea* (compositae), pistil bends and exposes the stigma on being touched by insects. In *Salvia,* versatile anthers and other balancing features help in dusting of insects with pollen.

4. Ornithophily:

Pollination by birds is not so common. Humming birds, sun birds and honey eaters are some of the birds which visit flowers and bring about pollination. Such flowers are tubular, cup-shaped or urn shaped, bright in colour and produce large quantities of pollens and plenty of nectar.

5. Cheiropterophily:

Pollination by bats is known as cheiropterophily. These flowers open only at or after dusk. On blooming, flowers emit an odour and produce large quantities of nectar. Flowers are dull in colour. Bats, being nocturnal are attracted by the odour of the flowers.

6. Malacophily:

Snails and slugs visit certain flowers and may be playing a role their pollination. Malacophily is generally observed in plants like *Arisaema* (cobra plant) and arum lilies.

2.5.4. Post pollination development of male gametophyte:

2.5.4.1. Formation of male gametes:

Pollens will germinate if both pollen and stigma are of the plants belonging to the same species or genus. After pollination, the male gametophyte (germinating pollen) is usually at two cell stage. It contains a vegetative cell and a generative cell.

The generative cell contains large amount of protoplasm. It acquires a characteristic vermiform appearance. It divides mitotically to form two male gametes. Pollen grains absorb moisture from stigmatic surface and swells up. Intine layer protrudes out through germ pore forming pollen tube. The nucleus of vegetative cell (pollen tube nucleus) moves towards the tip of the **pollen tube**. This three celled structure represents the male gametophyte of Angiosperms. Thus the male gametophyte in Angiosperm is a much reduced structure.

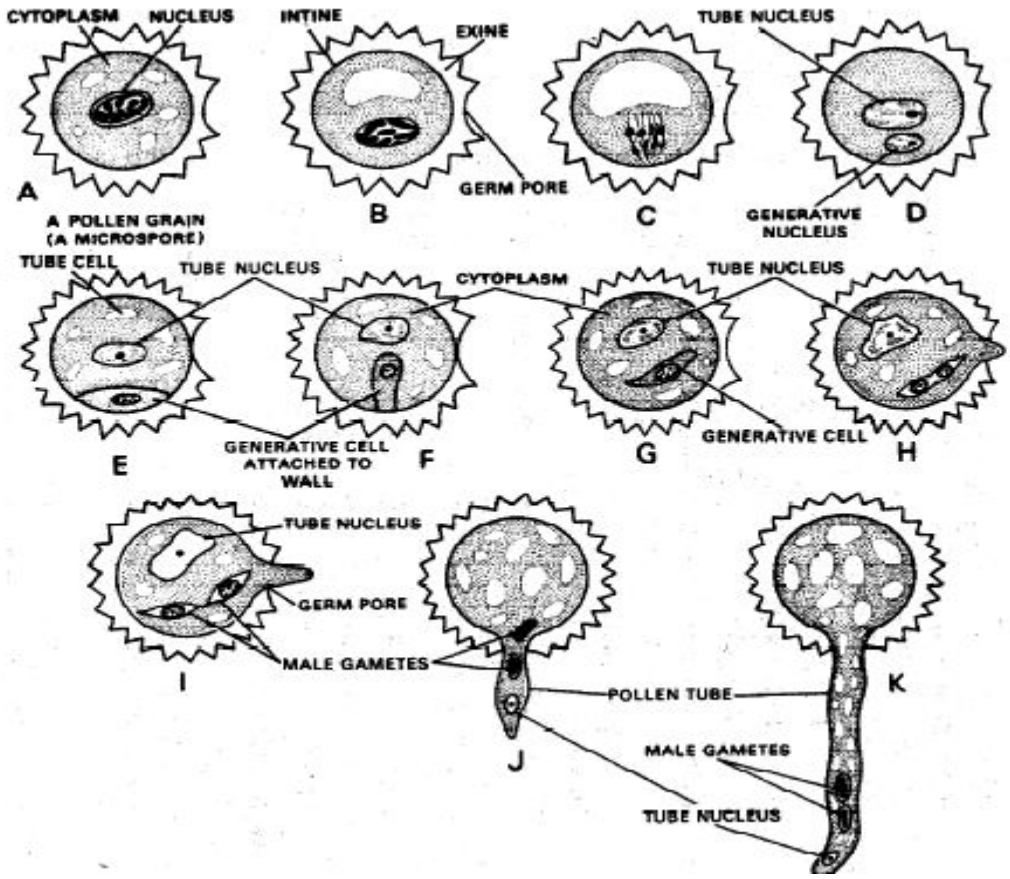


Fig.2.14 Developmental stages of male gametophyte

The male gametophyte depends upon the sporophyte. Pollen tube was discovered by **G.B. Amici** in ***Portulaca***. The function of pollen tube is to carry gametes. In the pollen tube, tube nucleus enters first which is vestigial and soon degenerates. The tube nucleus was earlier supposed to guide the passage of the pollen tube.

However, recent workers differ with the above opinion and consider it as a purely non-functional vestigial structure, based on the following facts:

1. In branched pollen tube, the tube nucleus remains in one of the tubes, but all the tubes grow normally.

2. It does not always occupy the position behind the tip of the pollen tube as in many cases it lies behind the male gametes.

3. In some cases, the growing pollen tube does not have any tube nucleus as it degenerates prior to the development of pollen tube.

2.6 SUMMARY

The stamen or microsporophyll consists of a filament, anther and a connective. Each anther consists of two anther lobes connected by a tissue known as connective. Each anther lobe contains two pollen sacs or microsporangia. Young anther shows a mass of undifferentiated cells surrounded by epidermis. The rows of hypodermal cells called microsporangial initials or archesporium becomes differentiated in each lobe of the anther.

Each microsporangial initial divides by periclinal division to form outer primary parietal cell and inner primary sporogenous cell. The primary parietal cell repeatedly divides to form the wall layers such as: 1-Epidermis, the outermost layer, 2- Immediately below the epidermis is endothecium; the cells are radially elongated and have fibrous thickenings. These cells are hygroscopic in nature and help in dehiscence, 3- Usually one to three middle layers below the endothecium. They become crushed at the time of meiotic division in the pollen, 4- The cells of the innermost layer of the wall possess dense protoplasm and the food reserve known as tapetum and it serves as a nutritive layer for the developing microspores. The tapetum may be glandular or amoeboid based on the behaviour of the cells during microsporogenesis. The inner primary sporogenous cells undergo several divisions to form microspore mother cells. Each microspore mother cell divides meiotically to produce four microspores or pollen grains having half (n) number of chromosomes.

Each pollen grain is unicellular and uninucleate structure having two layers as outer exine with spinous outgrowth or different types of ornamentation and inner thin, delicate intine made up of cellulose. The exine at some places is very thin. These thin points are known as germ pores.

The microspore is the first cell of the male gametophyte which starts germinating while it is still within the microsporangium or pollen sac. The nucleus of the microspore divides to form a generative nucleus and a tube or vegetative nucleus. The cell wall is formed resulting in two unequal cells called generative cell and vegetative cell. The generative cell is lenticular or spindle-shaped. Generally, the microspore is shed in the two-celled condition for pollination. The two celled pollen grain on the stigma of a flower becomes three celled as a result of the division of the generative cell into two male gametes. The pollen grain absorbs water and the intine grows out through a germ pore to form a pollen tube, two male gametes travel down into the style ovary and finally to embryo sac.

2.7 GLOSSARY

Androecium: (Gk. Andros, male), the stamens, which bear the filament and the anther.
Angiosperms: The flowering plants in which ovules are enclosed inside the ovary.
Dehiscence: (L.de, down; hiscence, split open), the opening of an anther, fruit, or other structure, which permits the escape of reproductive bodies contained within.
Exine: The outer wall layer of a microspore or pollen grain.
Intine: The inner wall layer of a microspore or pollen grain.
Microsporangium: A sac or chamber inside which microspores are produced.
Microspore: In heterosporous plants, smaller spores that develop into a male gametophyte.
Microsporocyte: The microspore mother cell, a cell in which meiosis will occur, resulting into 4 microspores.
Pollen Grain: It is a microspore which gives rise to male gametophyte.
Pollen Tube: A tube formed after germination of the pollen grain.
Pollen Sac: A cavity in the anther that contains the pollen grains.
Pollination: The transfer of pollens from the anther to stigma by insects, birds, animals in general.
Sporangium (pl. Sporangia, Gk. Spora, seed, + angeion, vessel) a hollow sac like structure in which spores are produced.
Spore: (Gk. spora, seed), the product of meiosis i.e. the reproductive, asexual cell, usually unicellular, and equipped with starchy reserve products, capable of developing into a gametophyte.
Sporocyte: A spore mother cell (2n) that undergoes meiosis and produces (usually) 4 haploid cells (spores).
Sporophyte: The spore-producing, diploid (2n) phase in a life cycle.
Tapetum: (Gk. tapes, a carpet), is the innermost layer of the wall of sporangium which is nutritive.

2.8 SELF ASSESSMENT QUESTIONS

2.8.1. Multiple Choice Questions

1. First cell of male gametophyte of angiosperms is:
a. microsporangium b. nucellus
c. microspore d. stamen

2. When pollen of a flower is transferred to the stigma of another flower of the same plant, the pollination is referred to as:
a. autogamy b. geitonogamy
c. xenogamy d. allogamy

3. In an angiosperm, how many microspore mother cells are required to produce 100 pollen grains?

a. 75 b. 100
c. 25 d. 50

4. Who is considered as **Father of Palynology?**

a. P.K. Nair b. Erdtman
c. Nawaschin d. Strassburgur

5. Ubisch bodies are found in

a. Amoeboid tapetum b. Glandular tapetum
c. Both a and b d. None of the above

6. Anemophily type of pollination is found in

a. *Salvia* b. bottle brush
c.*Valisnaria* d. coconut.

7. Compound Pollen grains are present in:

a. *Chenopodium* b. *Parthenium,*
c. *Ambrosia* d. *Drosera.*

8. In angiosperm all the four microspores of tetrad are covered by a layer which is formed by

a. pectocellulose b. callose
c. cellulose d. sporopollenin

9. Which one of the following is surrounded by a callose wall?

a. male gamete b. egg
c. pollen grain d. microspore mother cell.

10. Male gametes in angiosperms are formed by the division of

a. generative cell b. vegetative cell
c. microspore mother cell d. microspore

2.8.1 Answers key:

1. (c), 2. (b), 3. (c), 4. (b), 5. (b), 6. (d), 7. (d), 8.(a), 9. (d), 10. (a).

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- Heslop –Harrison, J. 1972, *Sexuality of angiosperms* pp133-289, In: Steward, F.C. (ed.), plant Physiology Vol. 6C, Academic press, New York

- Nair, .P. K.K. *Pollen Morphology of the Angiosperms: A Historical and Phylogenetic Study*. Scholar Publishing House and Vikas Publications, Delhi

2.10 SUGGESTED READINGS

- The Embryology of Angiosperms S.S. Bhojwani and S.P. Bhatnagar, Vikas Publishing House Pvt. Ltd, Noida, UP
- College Botany Vol II S.Chand Publication, New Delhi
- A Text book of Botany Singh Pande and Jain, Rastogi Publication, Meerut
- Wikipedia.org
- htttp://www.Biology

2.11 TERMINAL QUESTIONS

Q1. Draw well labeled diagram of T.S. Anther.
Q2. Write descriptive note of Tapetum and its function
Q3.Comment on Anther wall
Q4. What do you understand by microsporogenesis.
Q5. Differentiate between self and cross pollination
.Q6. Write short note on the following
(a) chireptrophilly
(b) polyspory
(c)Anemophilly
(d)Rare features of pollen
Q7. Write descriptive note on micro-gametogenesis.

UNIT-3: DEVELOPMENT OF FEMALE GAMETOPHYTE

3.1 OBJECTIVES

After going through this unit students will be able to answer the following questions.

- What is female gametophyte?
- Where does female gametophyte develop?
- What is the role of female gametophyte in flowering plants?
- What is megasporogenesis?
- What is megagametogenesis?
- Why embryo sacs are classified as monosporic, bisporic or tetrasporic?
- What are different patterns of female gametophyte development?

3.2 INTRODUCTION

Life cycle of vascular plants exhibits an alternation of generation that involves a multicellular haploid gametophyte and multicellular diploid sporophyte and both differ morphologically as well functionally. Haploid gametophyte is represented by 'n' number of chromosomes while diploid sporophyte is represented by '2n' number of chromosomes. Role of these gametophytic and sporophytic generations in the life cycle of plant is very important.

The major function of sporophytic generation is to produce spores which are haploid (n) as a result of meiosis (reduction division).

The major function of gametophytic generation is to produce haploid gametes.

We can say that **sporogenesis** is the start of sexual reproduction during which specialized cells known as spore mother cells, undergo meiosis and give rise to haploid **spores**. This process occurs within the sporangium. Spores undergo a process of cell proliferation and differentiation resulting into multicellular gametophytes which then produce the gametes (**gametogenesis**). Male and female gametes (sperm and egg) fuse to form the zygote, followed by embryo development and then giving rise to the sporophyte, thereby completing the life cycle (Gifford and Foster, 1989).

- Diploid sporophyte produces haploid spores as a result of reduction division (meiosis).
- Spores develop into gametophytes.
- Gametophytes produce haploid gametes.
- The fusion of female gamete (egg) and male gamete (sperm) gives rise to the zygote.
- Zygote is the beginning of diploid sporophyte.

Flowering plants or angiosperms are **heterosporous** because here sporophytes (diploid) produce two types of spores known as microspores and megaspores which develop into two types of gametophytes. [Plants which produce only one type of spores are called **homosporous**].

The one, that are smaller in size are called microspores. During microsporogenesis, diploid microspore mother cells (MMCs) give rise to haploid microspores as a result of meiotic division which then undergo microgametogenesis and develop into male gametophytes.

The other, that are larger in size are called megaspores. During megasporogenesis, diploid megaspore mother cells (MMCs) undergo meiotic division and haploid megaspores are formed which develop into female gametophytes as a result of megagametogenesis (Gifford and Foster, 1989).

In angiosperms gametophytes develop within sporophytic tissues means male gametophyte develops within the anther of stamen (McCormick, 1993, 2004) and female gametophyte (the embryo sac) develops within the ovule which is found within the ovary of carpel.

In the previous unit you must have studied about the male gametophyte. So you will be aware of structure of anther, microsporogenesis as well as the development of male gametophyte in angiosperms. By going through that unit it must be clear to you that the pollens or microspores develop by reduction division in microspore mother cell within the microsporangium and these microspores represent the first cell of male gametophyte.

Main focus of this unit is the development of female gametophyte. As the female gametophyte develops within the ovule, so this unit also includes ovule development, structure and types of ovules in addition to the development of female gametophyte.

Ovules as you have read, are enclosed by the ovary wall. The part of the carpellary tissue to which the ovules are attached is called **placenta** and the arrangement of ovules in the ovary is described as **placentation.**

Ovule also known as megasporangium is the place of formation of the megaspores and the female gametophyte. The female gamete after fertilization produces the embryo while the entire megasporangium with its enclosed structure becomes the seed and the progenitor of the next generation. The angiosperm female gametophyte is critical for plant reproduction.

3.3 OVULE

On the whole, angiosperm ovules are relatively small as compared with the ovules of gymnosperms and often develop in large numbers from the placentae of the ovary. The ovule at first arises as a primordium on the placenta in the cavity of the ovary by the localized periclinal divisions of the hypodermal cell layer. Later due to meristematic activity of the cells of ovular primordium, the protuberance becomes prominent and grows into a mass of tissue, **the young nucellus (**Fig 3.1, A**)**. As the nucellus enlarges, growth may be unequal, resulting in various degrees of curvature of the main body of the ovule. The type of development illustrated in Fig 3.1A leads to the common anatropous type of ovule in which the micropyle become directed inwardly towards the point of attachment of the funiculus. If the ovule develops in an erect position, it is designated as orthotropous ovule. The initials of two integuments arise as rim-like outgrowths from the surface cells of the nucellus. The inner integument which is usually formed first, initiates from the epidermal layer and the outer integument is initiated either dermally or sub-epidermally (Fig 3.1 A, B). With the differentiation of integuments the ovule begins to curve and by the megaspore tetrad stage it assumes its final shape. Although the integuments initiate later they grow faster than the nucellus. The integuments soon cover the nucellus, leaving a small opening at the tip, the micropyle. Figure 1 is showing the stages of ovule development.

3.3.1 Structure of ovule

The **megasporangium or ovule** consists of **nucellus** and its protective coats, the **integuments**. It is attached to the placenta (on the inner wall of ovary) by a stalk called **funiculus (funicle)** and the point of attachment of the body of the ovule to the funicle is called **hilum.**

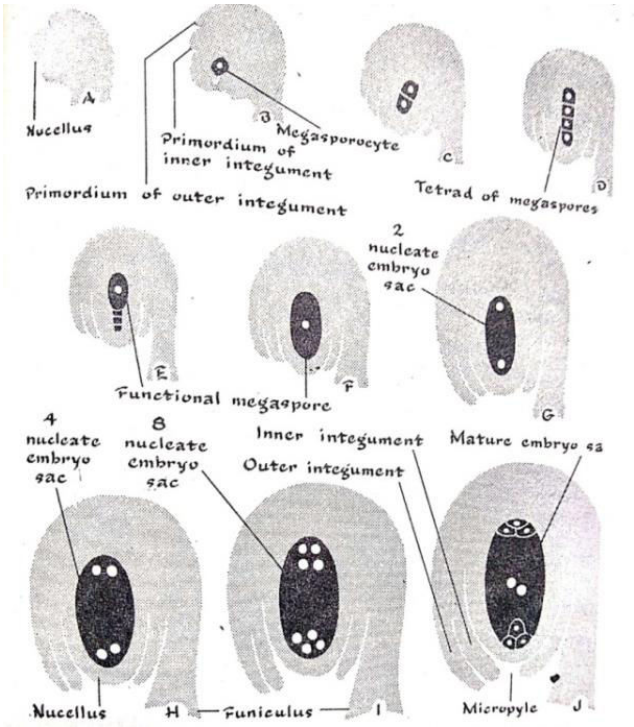


Figure 3.1*- Development of an angiosperm ovule, beginning with the formation of the integuments and the single megasporocyte (A-B), continuing through the formation of megasores (C-E), and concluding with the successive stages in development of the embryo sac (F-J). (Taken from Comparative Morphology of Vascular Plants by A.S. Foster and E.M. Gifford: W.H.Freeman and Company, 1958)*

A mature ovule consists of nucellus enveloped almost completely by one or two sheaths, known as **integuments**, except a small opening at the apical end. This opening is known as **micropyle**. The basal region of the ovule where it is attached to the placenta by funicle, is called **chalaza** and so this side is known as chalazal end. Its opposite end is termed as micropylar end having the main passage for the entry of the pollen tube into the ovule. In the nucellus, **female gametophyte** is present, also known as **embryo sac** (Figure 3.2)**.**

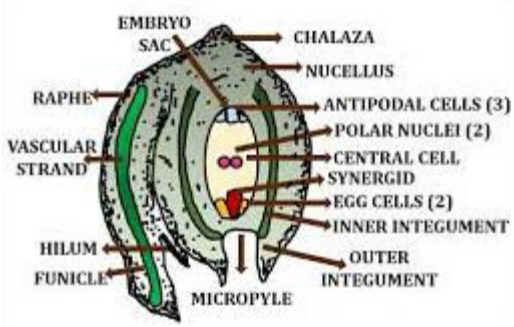


Fig 3. 2- *Structure of ovule*

Nucellar tissue is parenchymatous and represents the wall of the megasporangium. The nucellus is mostly consumed by the developing embryo sac. Each ovule has only one nucellus. However, two nucelli may occur as abnormality within a common fold of integuments as has been observed in *Aegle marmelos*.

The ovule with a **single integument** is called **unitegmic,** and with **two integuments** is called **bitegmic.** In some insectivorous plant (*Drosera*) the ovule is without integument (**ategmic**).

Parts of the ovule:

1. Funicle : A stalk by which ovule is attached to the placenta
2. Nucellus: the body of ovule
3. Integument: the protective covering of nucellus
4. Micropyle: small opening formed by integument(s) over nucellus
5. Chalaza: basal part of the ovule
6. Hilium: region where ovule attached with funicle
7. Embryo sac: female gametophyte located in the nucellus

3.3.2 Types of ovule

Dear students, if we consider the position of the micropyle with respect to the funiculus, ovules are mainly of six types (Figure 3.3).

A. Orthotropous
B. Anatropous
C. Campylotropous
D. Amphitropous
E. Hemianatropous
F. Circinotropous

A.Orthotropous ovule:

If the position of micropyle, chalaza and the funiculus is in one straight line, then it is known as orthotropous ovule. Because there is no turn or curvature in it and it is straight and upright, so can also be called as **atropous (**without curvature**).** It is found in Polygonaceae and Piperaceae.

B. Anatropous ovule:

Anatropous ovule, unlike orthotropous ovule is completely inverted. This all happens because of the unilateral growth of the ovule. As a result of this, the position of micropyle and chalaza is in one line but funiculus lies parallel to it. Here micropyle lies very close to the base of the funicle. Majority of angiosperms exhibit this type of ovule.

C. Amphitropous ovule:

In amphitropous ovule the curvature of the ovule is very deep and affects the nucellus as well as the embryo sac so it bent. This results into 'horse shoe' like appearance of the ovule. Example- in Alismaceae and Butomaceae.

D.Campylotropous ovule:

Curvature is also seen in campylotropous ovule but it is less than that in anatropous ovules. Micropylar part only is bending downwards. Due to this curve micropyle and chalaza could not lie in straight line and the position of the funicle is at right angle to the chalaza. Example- in Chenopodiaceae and Capparidaceae.

E.Hemianatropous ovule:

Unlike campylotropous ovule, in hemianatropous or **hemitropous** ovule, the position of the funicle is at right angle to the nucellus and the integuments. Position of micropyle and chalaza, is in one plane. Example- in *Ranunculus*, *Nothoscordum*, and *Tulbaghia*.

F. Circinotropous ovule:

Some members of the Plumbaginaceae exhibit a very peculiar type of ovule. Here due to rapid and unilateral growth, the ovule first become anatropous then turned over completely. As a result of this continuous growth and curvature, the micropylar end again points upwards. Such special type of ovule has been named as circinotropous (Archibald, 1939). Example- in *Opuntia*,

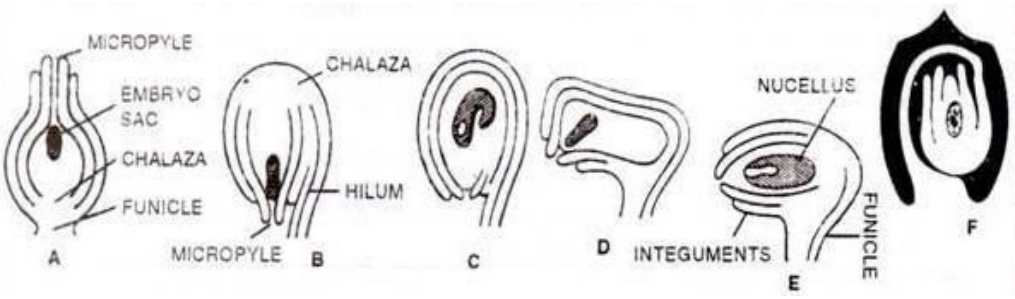


Figure 3.3: *Different types of ovules: A. Orthotropous ovule B. Anatropous ovule C. Amphitropous ovule D. Campylotropous ovule E. Hemianatropous ovule F. Circinotropous ovule*

Considering the extent of development of the nucellus and position of sporogenous cell, ovule can also be categorized as

1. Tenuinucellate type
2. Crassinucellate type

1. Tenuinucellate type:

The archesporial cell in nucellus is hypodermal in origin. When it directly functions as the megaspore mother cell, the position of this megaspore mother cell (sporogenous cell) is also hypodermal. So the nucellar tissue around it also remains single-layered. Such ovules, where the position of sporogenous cell is hypodermal and the nucellar tissue around it remains single-layered, are called **tenuinucellate** (Figure 3.4) type of ovule.

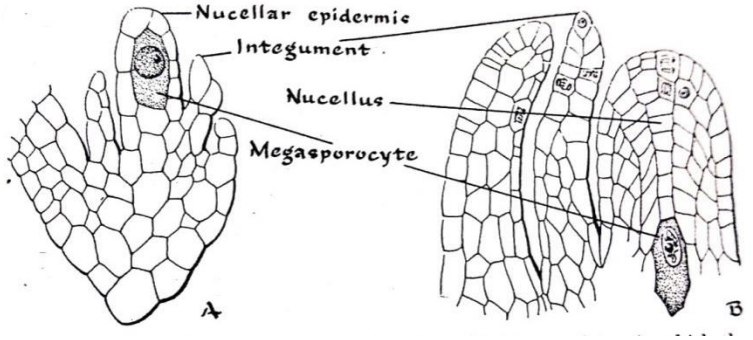


Figure 3.4: *A. Tenuinucellate type of ovule in Orchis maculates, in which the megasporocyte occurs directly below the nucellar epidermis; B.Crassinucellate type of ovule in Quisqualis indica (note deeply embedded position of megasporocyte and the active periclinal divisions in the nucellar epidermis). [Adapted from An Introduction to the Embryology of Angiosperms by P. Maheshwari. N.Y.: McGraw-Hill Book Company, Inc., 1950]*

2. Crassinucellate type:

Unlike tenuinucellate type, the hypodermal archesporial cell divides into two cells by means of transverse division. The outer one is parietal cell and an inner one is sporogenous cell. The parietal cell may either remain undivided or undergo a few divisions. These divisions are both periclinal as well as anticlinal. As a result of this the sporogenous cell becomes embedded in the massive parietal tissue. The sporogenous cell may also be embedded in the massive nucellus by divisions in the nucellar epidermis instead of division in archesporial cell. All such ovules where the sporogenous cell becomes sub-hypodermal, by either above two means, are called **crassinucellate** (Figure 3.4).

Tenuinucellate ovule: where the sporogenous cell is hypodermal and the nucellar tissue around it remains single layered

3.4 DEVELOPMENT OF THE FEMALE GAMETOPHYTE

In general female gametophyte development occurs within the ovule (megasporangium) and completes in two phases:

1. Megasporogenesis
2. Megagametogenesis

Development of the megaspore from megaspore mother cell is known as **megasporogenesis** and a process of cell proliferation and differentiation in megaspore to develop into multicellular female gametophyte is known as **megagametogenesis.**

Angiosperm plants follow many different patterns of female gametophyte development. The common developmental pattern exhibited by over 70% of flowering plants is referred to as the Polygonum-type. Example- Poaceae (e.g., maize, rice, wheat), Phaseoleae (e.g., beans, soyabean), Brassicaceae (e.g., *Brassica*), Malvaceae (e.g., cotton), and Solanaceae (e.g., pepper, tobacco, tomato, potato, petunia) and most apomictic species (Maheshwari 1950, Willemse and van Went 1984, Huang and Russell 1992). It is known as Polygonum-type because it was first described in *Polygonum divaricatum* (Strasburger, 1879; Maheshwari, 1950).

Megasporogenesis

"Development of the megaspore within the ovule (megasporangium) from megaspore mother cell is known as megasporogenesis."

At the micropylar end, one of the hypodermal cells in the nucellus differentiates and functions as the **archesporial cell** (archesporium). Being large in size, having dense cytoplasm and large nucleus, it becomes more prominent than its surrounding cells. Therefore is easily distinguishable from the other cells.

Depending on the basis of position of sporogenous cell, ovule can be categorized into two types-1.tenuinucellate type and 2.crassinucellate type (described earlier).

So here **in tenuinucellate type of ovule**, the archesporial cell directly functions as megaspore mother cell (MMC) and **in crassinucellate type of ovule** the archesporial cell do not directly behave as MMC and instead of that it divides periclinally into two cells. An outer primary parietal cell (towards epidermis) and an inner primary sporogenous cell. Now this primary sporogenous cell functions as the megaspore mother cell.

Megaspore mother cell, also known as **megasporocyte** is diploid in nature, representing the last cell of sporophytic generation. It undergoes meiosis i.e. reduction division. As a result of this four haploid megaspores (tetrad) are formed. Tetrad of megaspores may be arranged in linear, T-shape, isobilateral or tetrahedral patterns. T-shaped tetrad arises due to vertical division in the micropylar dyad cell and transverse division in the chalazal dyad cell. In case of linear arrangement which is most common, after the first meiotic division, the wall is laid down transversely, forming a dyad. The second meiotic division in the two dyad cells is also transverse. In this way a row of four haploid megaspore cells (**linear tetrad)** is formed (Figure 3.5 and 3.6).

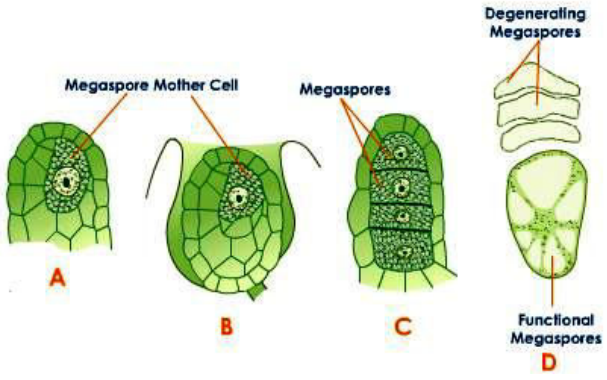


Figure 3.5-*Formation of 4 haploid megaspores from diploid megaspore mother cell*

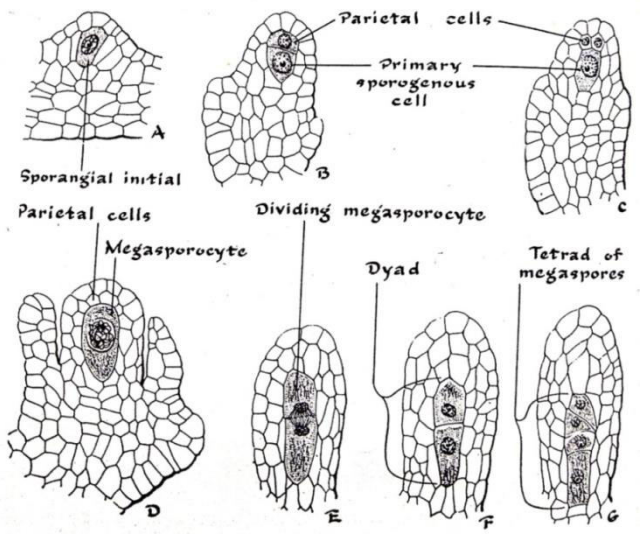


Figure 3.6. *Megasporogenesis in the ovule of Hydrilla verticillata. A. hypodermal sporangial initial. B. Periclinal division of sporangial initial into parietal cell and primary sporogenous cell C. results of anticlinal division of parietal cell D. megasporocyte situated below the layer of wall cells E-F. formation of dyad cells G. linear tetrad of megaspores (Adapted from An Introduction to the Embryology of Angiosperms by P. Maheshwari. N.Y.: McGraw-Hill Book Company, Inc., 1950)*

In the linear tetrad, **the lowermost megaspore** (the chalazal megaspore) enlarges and becomes **functional,** while rest three megaspores of tetrad do not participate in the formation of female gametophyte and degenerate.

The functional megaspore now forms **the female gametophyte (embryo sac)**.

If you remember the microsporogenesis where a haploid microspore was said to be the first cell of male gametophyte, similarly here **megaspore is** known as **the first cell of female gametophyte**. In angiosperms the development of the female gametophyte is completely **endosporous** means within the megaspore.

Megagametogenesis

Megagametogenesis could be defined as a process of cell proliferation and differentiation in megaspore cell to develop into multicellular female gametophyte.

Initially, the surviving megaspore undergoes free nuclear divisions (without cytokinesis) resulting into a multinucleate coenocyte. Subsequently, development of cell walls around these nuclei leads to the development of a female gametophyte (Figure 3.5).

In case of Polygonum-type of female gametophyte, the single functional (surviving) megaspore increases in size. After enlargement it undergoes two rounds of mitosis without cytokinesis. As a result of which a four-nucleate coenocyte formation takes place having two nuclei at each pole and all these nuclei divide again third time. After third mitosis eight-nucleate coenocyte formation takes place having four nuclei at each pole.

Now one nucleus from each pole migrates toward the center of the developing female gametophyte known as the polar nuclei which may or may not fuse together either before or after the entry of pollen tube.

Ultimately an eight nucleated structure consisting of three antipodal cells, two polar nuclei (central cell), two synergid cells and one egg cell is formed.

The central cell having two identical haploid nuclei and is therefore called as homodiploid. The other all cells inherit single haploid nuclei (Figure 3.7).

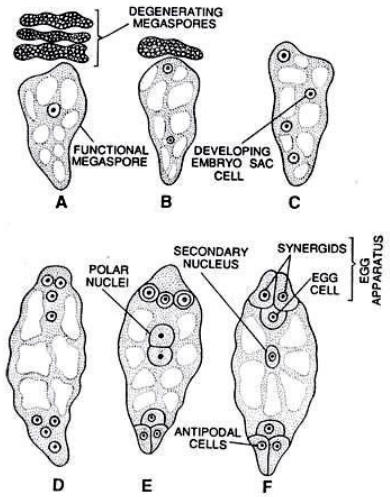


Figure 3.7*- Development of female gametophyte of normal type*

Figure 3.8 and Figure 3.9 showing megasporogenesis (the diploid megaspore mother cell undergoes meiosis and gives rise to haploid megaspores) and megagametogenesis (one of the megaspores develops into the mature female gametophyte), respectively in Arabidopsis.

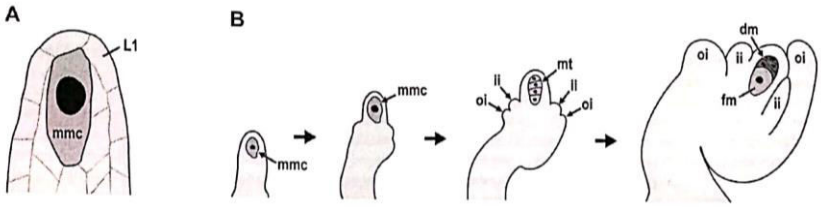


Figure 3.8*-Megasporogenesis in Arabidopsis.*

A. Apical region of a young, finger like ovule primordium. The megaspore mother cell forms from a sub-epidermal cell at the distal end of the ovule primordium. L1 is the outer layer of cells.

B. Steps of megasporogenesis. Ovule promordia arise as finger-like projections from the placenta. The megaspore mother cell undergoes meiosis and forms four megaspores. Three of the megaspores undergo cell death. The chalazal-most megaspore survives, becomes the functional megaspore and undergoes megagametogenesis.

Black circles/ovals represent nuclei.dm: degenerating megaspores; fm: functional megaspore; ii: inner integument; L1: epidermal layer of the ovule promordium; mmc: megaspore mother cell; mt: meiotic tetrad; oi: outer integument.

Source:Drews and Koltunow(2011)

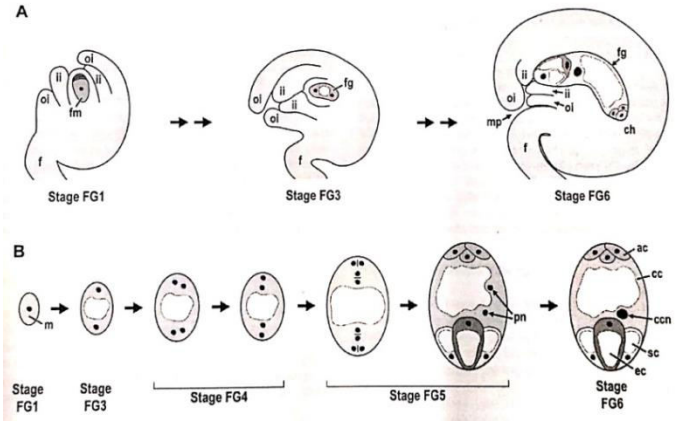


Fig. 3.9*-Megagametogenesis in Arabidopsis*

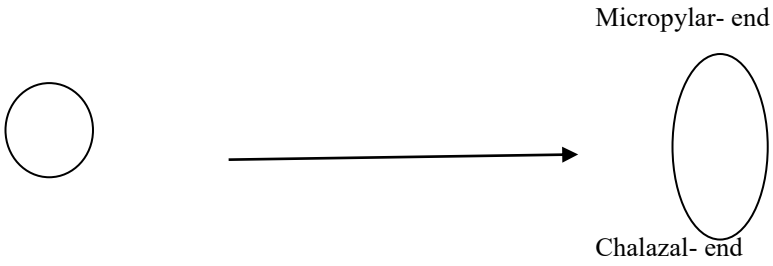
A. Steps of megagametogenesis emphasizing development within the ovule.

B. Stages of megagametogenesis (Christensen et al. 1998). The megaspore contains a single nucleus (stage FG1). The nucleus undergoes two rounds of mitosis, producing four-nucleate coenocytes, with two nuclei at each pole separated by a large central vacuole (stage FG4). During a third mitosis, phragmoplsta and cell plates form between sister and non-sister nuclei and the nuclei become completely surrounded by cell walls (stage FG5). During cellularization, the polar nuclei migrate towards the centre of the female gametophyte and fuse before fertilization. These events produce a seven-celled structure consisting of three antipodal cells, one central cell, two

synergids, and one egg cell. If the female gametophyte is not fertilized, the antipodal cells eventually degenerate (stage FG7, not shown).
White areas represent vacuoles and black circles/ovals represent nuclei.
Ac: antipodal cells; cc: central cell; ccn: central cell nucleus; ch: chalazal region of the ovule; ec: egg cell; f: funiculus; fg: female gametophyte; fm: functional megaspore; ii: inner integument; m: megaspore; mp: micropyle; oi: outer integument; pn: polar nuclei; sc: synergid cells.
Source: Drews and Koltunow (2011)

Steps of development of the female gametophyte or embryo sac in detail:

The development of embryo sac begins as the functional megaspore elongates. You know that in most cases the lowermost megaspore (chalazal) of the linear tetrad becomes functional and rest three degenerates. The elongation is largely along the micropylar-chalazal axis.



Functional megaspore Embryo sac

The first nuclear division (post- meiotic mitosis) in the megaspore is not followed by wall formation. A large central vacuole appears between the two daughter nuclei. Initially there is no vacuole in the cytoplasm of the megaspore but later small vacuoles appear which may fuse to form large vacuole. As the vacuole expands, the nuclei pushed toward opposite poles of the cell. In this way each pole has one nucleus. Now both the nuclei divide twice, forming four nuclei at each pole. All the divisions are mitotic and without wall formation.

Dear students you can understand that at this stage all the eight nuclei are present in the common cytoplasm because after haploid megaspore formation all the nuclear divisions are not followed by cell wall formation. After the last nuclear division (when there are eight nuclei) the cell undergoes appreciable elongation, so that it looks like sac. The picture must be clear in your mind that out of these eight nuclei, four are at the micropylar-end and four at the chalazal-end.

Finally the embryo sac becomes organized. Three nuclei at the micropylar-end of the embryo sac organize into **egg apparatus** and the fourth one is left free in the cytoplasm of the central cell and moves toward the centre as the upper polar nucleus. The egg apparatus

(only upper portion) is attached to the wall of the embryo sac at the micropylar-end, its major portion is surrounded by the middle cell. In the **egg apparatus**, the middle cell is **egg cell (round, also known as ovum or oosphere)** and the rest two side cells are **synergids** which are flask shaped. Only the synergid cells are in direct contact with the wall of the embryo sac. The central egg cell is situated in such a way that its upper portion is slightly below the apices of the synergids and seems hanging between and below them. All the three cells are of same length therefore the egg cell extends a little more towards the centre in comparison to the synergids. Out of the four nuclei at the chalazal-end of the embryo sac, three nuclei forms three **antipodal cells** and the fourth one moves toward the centre as the lower polar nucleus.

You have read that one nucleus from each pole moves to the center of the embryo sac, here they may fuse forming the **fusion or secondary nucleus**. The secondary nucleus (if fusion occurred between two polar nuclei) or two polar nuclei (if there is no fusion) remains at the center.

> The haploid nucleus of the **megaspore divides mitotically** (non-reductional division) which organize in a definite manner within the embryo sac. Three nuclei at the micropylar-end, three at the chalazal-end and the remaining two migrate to the centre of the embryo sac. Three nuclei at the micropylar-end organize into an egg apparatus. The central large cell of the egg apparatus is called egg cell (female gamete) which is partially surrounded by two lateral synergid cells. Three nuclei of the chalazal-end form antipodal cells. The two nuclei which migrates to the centre, called polar nuclei. These polar nuclei later fuse to form a single diploid secondary nucleus (a central cell).

These events result in a mature **seven celled structure** called **female gametophyte or embryo sac** consisting of three antipodal cells, one central cell having two polar nuclei, two synergid cells, and one egg cell. **Since, this type of embryo sac develops from a single megaspore and has eight nuclei, it is said to be monosporic-8-nucleate embryo sac** or **Polygonum -type** of embryo sac. Throughout development, the female gametophyte exhibits a polarity along its chalazal-micropylar axis.

Important Points

- The female gametophyte (embryo sac) is 7-celled (mostly), 8-nucleate structure having **three cells** of egg apparatus (two synergid cells and one egg cell) at the micropylar-end, **three cells** (antipodal cells) at the chalazal-end and **one cell** (centre cell) in the centre having two polar nuclei.

-This type of embryo sac is designated as the **Polygonum-type,** formed as a result of three divisions in functional megaspore.

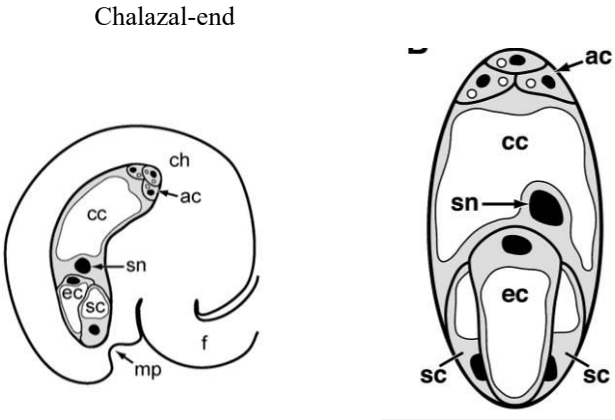
-This mode of embryo sac development occurs in the majority type of flowering plants. According to Davis (1966), about 81 per cent of the families show Polygonum-type of embryo sac development.

- Cells of the egg apparatus and the antipodal cells are uninucleate and haploids whereas the central cell is binucleate or diploid.

The entire embryo sac is enclosed within **diploid sporophytic tissues called integuments,** which will constitute the seed coat in the mature seed.

Detailed structure of the mature female gametophyte

Transmission electron microscopic structure of mature female gametophyte in *Arabidopsis* (Figure 3.10) has been described (Mansfield *et al*., 1991; Murgia *et al*., 1993; Kasahara *et al*., 2005; Kagi *et al*., 2011). The mature female gametophyte in *Arabidopsis* is ~105 µm long and ~25 µm wide and is Polygonum-type.



A: Ovule **B: Female gametophyte**

Fig. 3.10. A: *The Arabidopsis Female gametophyte within the ovule.* ***B****: The mature female gametophyte. The gray area represents cytoplasm, the white area represents vacuoles and the black area represents the nuclei. ac: antipodal cells; cc: central cell; ch: chalazal region of the ovule; ec: egg cell; f: funiculus; mp: micropyle; sc: synergid cell; sn: secondary nucleus. Picture courtesy of Yadegari and Drews (2004).*

The egg cell and central cell are polarized such that the nuclei of both cells lie very close to each other (Drews and Koltunow 2011). This feature is very important during double fertilization because these two nuclei are involved or we can say are the targets of the two male nuclei.

The cell wall is either absent or discontinuous at the junction of egg, synergid and central cell so that the plasma membrane of these cells is in direct contact with each other (Mansfield *et al*., 1991; Kasahara *et al*., 2005). This discontinuation or absence of cell wall in these regions provide direct access of the male gametes or sperm cells to the fertilization targets i.e. egg nucleus in the egg cell and secondary nucleus in the central cell because one of the synergid cells receives two male gametes from the growing pollen tube (Figure 3.11).

The synergid cell wall is further modified. At the micropylar site it is thickened and extensively invaginated, forming a structure known as filiform apparatus. Formation of filiform apparatus by means of invaginations in the cell wall of synergids helps in increasing the surface area of the plasma membrane in this region. It also contains a high concentration of secretory organelles. It is supposed that it may facilitate transport of

substances into and out of the synergid cells (Drews and Koltunow 2011) (Figure 3.11). Findings of cytological staining properties in other plant species concluded that the filiform apparatus is composed of cellulose, hemicellulose, pectin, callose, and proteins.

Functions of filiform apparatus (Maheshwari 1950, Willemse and van Went 1984, Huang and Russell 1992, Punwani and Drews 2008)

It has at least two functions associated with the process of fertilization.

1. The filliform apparatus secretes pollen tube attractants in the synergid cells.

2. The filliform apparatus provides route for the entry of pollen tube in the synergid cell, suggesting that the filliform apparatus is important for pollen tube reception.

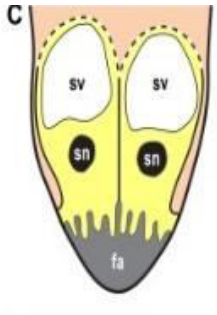


Fig. 3. 11-*The Synergid cells: The dashed lines of the synergid cells represent a discontinuous or absence of cell wall. Synergids are frequently observed to have elaborate wall projections, the filiform apparatus that extends into the nucellus, which may provide a mechanism for nutrient flow from the ovule to the embryo sac. fa, filiform apparatus; sn, synergid nucleus; sv, synergid vacuole*

(Photo courtesy: Drews and Koltunow, 2011)

Schematic development of female gametophyte, based on the model plant *Arabidopsis* is given in Figure 3. 12.

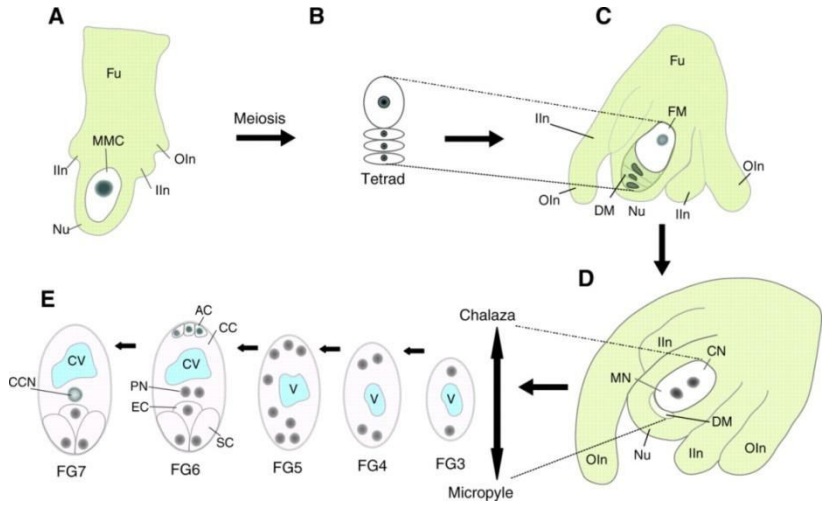


Fig. 3.12 *Schematic development of female gametophyte, based on the model plant Arabidopsis.*

(A) The megaspore mother cell (MMC) is surrounded by epidermal cells of the nucellus (Nu) prior to undergoing meiosis to generate four spores. At this stage, the formation of the outer (OIn) and inner (IIn) integuments has just initiated.

(B) Diagram of MMC asymmetric meiosis that generates four spores (tetrad). Three of these undergo programmed cell death. The proximal (chalazal) megaspore becomes the functiona lmegaspore (FM). (C) FG1 stage. The FM is teardrop-shaped andundergoes the first mitotic division.

(D) FG2 stage. The female gametophyte comprises two nuclei. The nucellus (Nu) is enclosed by the OIn, but not the IIn integuments.

(E) Stages FG3 to FG7. The female gametophyte comprises two nuclei, separated by a large vacuole (V), that undergo second and third mitotic divisions to generate the eight-nucleate mature embryo sac at the FG5 stage. Subsequent cellularization (FG6 stage) results in the formation of seven cells: two synergid cells (SC); one egg cell (EC); one central cell (CC) carrying two polar nuclei (PN); and three antipodal cells (AC). By FG7, the two polar nuclei have fused to form the central cell nucleus (CCN), and the antipodal cells degenerate.

CN, chalazal nucleus; CV, central vacuole; DM, degenerating megaspores; Fu, funiculus; IIn, inner integuments; MMC, megaspore mother cell; MN, micropylar nucleus; Nu, nucellus; OIn, outer integuments.

Source: Sundaresan, and Alandete-Saez. Development. 2010 137: 179-189; doi: 10.1242/dev.030346

The egg cell	→	The embryo
Two synergid cells	→	For pollen tube attraction
The central cell	→	The endosperm

Female gametophyte Polarity

Throughout the process of development, the female gametophyte exhibits a polarity along its chalazal-micropylar axis. We can say that the ovule and female gametophyte are polarized structures as shown in figure 1. The integuments form a pore called micropyle at the anterior pole and its chalazal pole is the posterior end that joins the funiculus (inverted position in anatropous ovule).

3.5 ALTERNATIVE PATTERNS OF FEMALE GAMETOPHYTE DEVELOPMENT

The Polygonum-type of female gametophyte development is the most common pattern. Many other patterns of development of female gametophyte also exist.

These different patterns are because of the variations in megasporogenesis and megagametogenesis both.

3.5.1. Patterns due to variation in megasporogenesis

Megasporogenesis among a**ngiosperms exhibit three main patterns**, referred to as **monosporic, bisporic, and tetrasporic** (Figure 3.13).

These three patterns differ mainly in - whether wall (cell plate) formation occurs after each meiotic division or not.

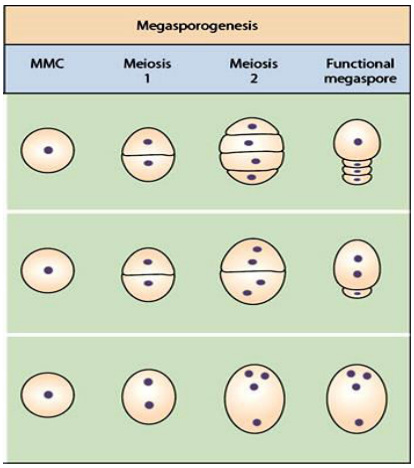
This decides the number of meiotic products that contribute to the formation of the mature female gametophyte, and also the pattern**.**

In the monosporic pattern, each phase of meiotic division is accompanied by wall formation, resulting in four uninucleate megaspores (**linear tetrad**). Only one becomes functional which forms the female gametophyte (embryo sac).

In the bisporic pattern, cell wall develops after meiosis I but not after meiosis II resulting in two binucleate cells. One of the dyad cells degenerates, resulting in a single functional

dyad cell possessing two megaspore nuclei. This functional **dyad cell** with two megaspore nuclei contributes towards the formation of female gametophyte.

In the tetrasporic pattern, neither first nor second meiotic division is accompanied by wall formation resulting in the formation of four megaspore nuclei inside the cell. **All four megaspore nuclei** participate in the formation of female gametophyte.



Monosporic pattern- one megaspore contributes to formation

Bisporic pattern - two megaspore nuclei (dyad cell) contribute to formation of the mature female

Tetrasporic pattern – All the four-megaspore nuclei contribute to formation of the mature female

Fig. 3.13- *Patterns of megasporogenesis*

3.5.2. Patterns due to variation in megagametogenesis.

Same general pattern as discussed above for *Arabidopsis* i.e. a phase of nuclear proliferation without cytokinesis followed by cellularization and differentiation is reported in majority of angiosperm species.

Variations arise because of:

-the number of nuclei within the megaspore that give rise to the female gametophyte (i.e. the type of megasporogenesis)

-the number of mitoses prior to cellularization

-the timing of fusion of the polar nuclei

-whether or not additional mitoses occur after cellularization.

(Maheshwari 1950; Willemse and van Went 1984; Haig 1990; Huang and Russell 1992; Yadegari and Drews 2004).

As in maize, exhibiting Polygonum- type of female gametophyte, the polar nuclei do not fuse until fertilization and the antipodal cells proliferate into 40 or more cells (Diboll and Larson 1966, Diboll 1968).

Therefore **depending on the basis of involvement of number of megaspore nuclei** in its formation, the embryo sac can also be called as: Monosporic, Bisporic and Tetrasporic

3.5.3 Types of Female gametophyte/Embryo Sac Development

Maheshwari (1950) has developed a classification of the various types and subtypes of embryo sac development in the angiosperms. His classification is fundamentally based on:
(1) The number of spores or spore-nuclei which enter into the formation of embryo sac
(2) The total number of nuclear divisions which occur during megasporogenesis and megagametogenesis and
(3) The number, arrangement and chromosome number of the nuclei in the mature embryo sac.

1. Monosporic Type:

Here megasporogenesis results in four well-defined megaspores, one of which gives rise to the embryo sac, rest three megaspores degenerate. Most commonly the megaspore farthest from the micropyle is functional. In this type of embryo sac all the nuclei are genetically identical because they are formed through mitosis of a single nucleus.
Monosporic embryo sacs are further divided into two types.

1. Polygonum type (8 nucleate)
2. Oenothera type (4 nucleate)

(i) Polygonum type (8 nucleate): As described earlier, it is formed by the chalazal megaspore of the tetrad and is eight nucleate. The mature Polygonum type of embryo sac comprises a 3-celled egg apparatus, three antipodal cells and a binucleate central cell means seven -celled, eight- nucleate monosporic embryo sac (Figure 3.14).

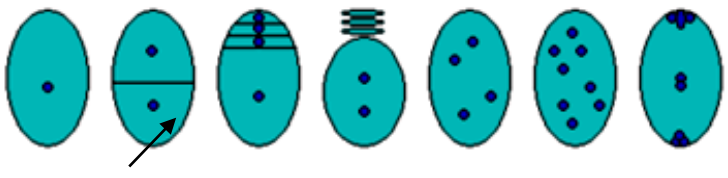
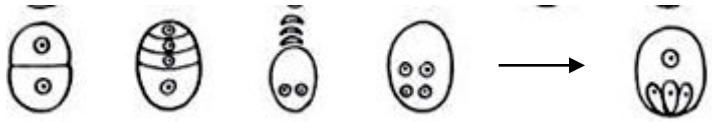


Figure 3.14- *Polygonum type embryo sac*

This type of embryo sac is the most common and is, therefore, commonly designated as the "Normal type." However, it is also designated as the Polygonum- type because it was reported for the **first time in *Polygonum divaricatum* by Strasburger (1879)**.

(ii) Oenothera type (4 nucleate): Oenothera type of embryo sac is derived from the functional micropylar megaspore of the tetrad and is four nucleate. The mature embryo sac consists of an egg apparatus and a uninucleate central cell (polar nucleus). Oenothera type of embryo sac is found in Onagraceae family.

Geert, in 1908, found that in *Oenothera lamarckiana* the embryo sac is usually formed by the micropylar megaspore of the tetrad, which undergoes only two nuclear divisions instead of the usual three occurring in the Polygonum type of embryo sac. In this way, 4 nuclei are produced which organize into the two synergids, the egg and a single polar nucleus. Since the third division is omitted and all the nuclei are situated in the micropylar part of the developing embryo sac, there is neither a lower polar nucleus nor any antipodal cells (Figure 3.15).



Micropylar megaspore

Fig. 3.15-*Oenothera type embryo sac*

The Oenothera type is of particular morphological interest because following double fertilization, the primary endosperm nucleus is *diploid* rather than triploid or polyploid as in other angiosperms.

2. Bisporic Type:

As described earlier, in this type of embryo sac as the name indicates **two megaspore nuclei** participate in its formation. After first meiotic division a dyad is formed by wall formation. Only one of the dyad cells undergoes the second meiotic division and the other one degenerates. In the functional dyad, cell division is not followed by wall formation and so both the megaspore nuclei (haploid) participate in the formation of the embryo sac. Each megaspore nucleus undergoes two mitotic divisions forming eight nuclei and mature embryo sac has the same organization like that of the Polygonum- type.

So you can say the bisporic embryo sacs are 8- nucleate and arise from one of the two dyad cells formed after meiosis I.

Tetrad formed as a result of meiosis in MMC, has four genetically different nuclei. **Being a derivative of two meiotic products, nuclei of a bisporic embryo sac are genetically different** (four nuclei are of one type and other four of a different type). A bisporic embryo sac was **first described in *Allium fistulosum* (Strasburger, 1879)** and has since been confirmed in several species of this genus.

Bisporic embryo sacs are also of two types.

1. Allium type
2. Endymion type

(i) Allium type (8 nucleate): In this type chalazal dyad cell participates in the formation of the embryo sac (Figure 3.16).

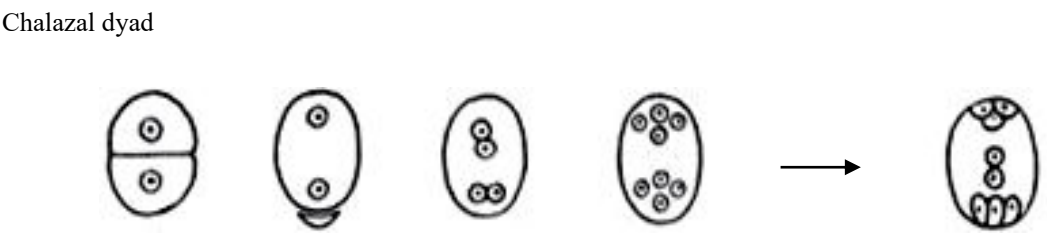


Fig. 3.16 *Allium type embryo sac*

(ii) Endymion type (8 nucleate): In this type micropylar dyad cell participates in the formation of the embryo sac (Figure 3.17).

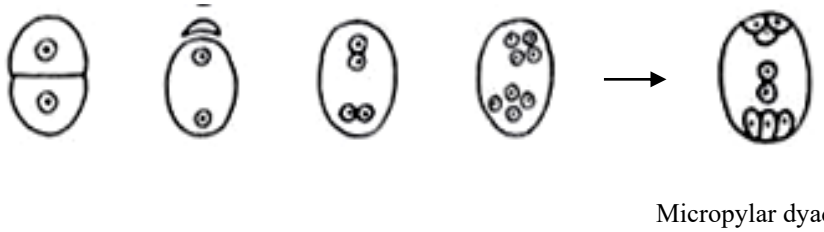


Fig. 3.17-*Endymion type embryo sac*

3. Tetrasporic Type:

This type of embryo sac development is remarkable because of the complete elimination of wall formation during meiosis and the participation of all **four megaspore nuclei** in the formation of the embryo sac. That's why it is called **tetrasporic embryo sac**. In this type neither of the meiotic division (first as well as second) is accompanied by wall formation so at the end of meiosis all the four haploid nuclei remain in a common cytoplasm forming a **coenomegaspore (four nuclei inside a cell)** and all the four nuclei of the coenomegaspore take part in the formation of embryo sac. This type of embryo sac is **more**

heterogenous than a bisporic one because all the four nuclei of the coenomegaspore, products of meiosis are genetically different. Three main types of patterns for embryo sac development can become clear by going through Figure 3.18.

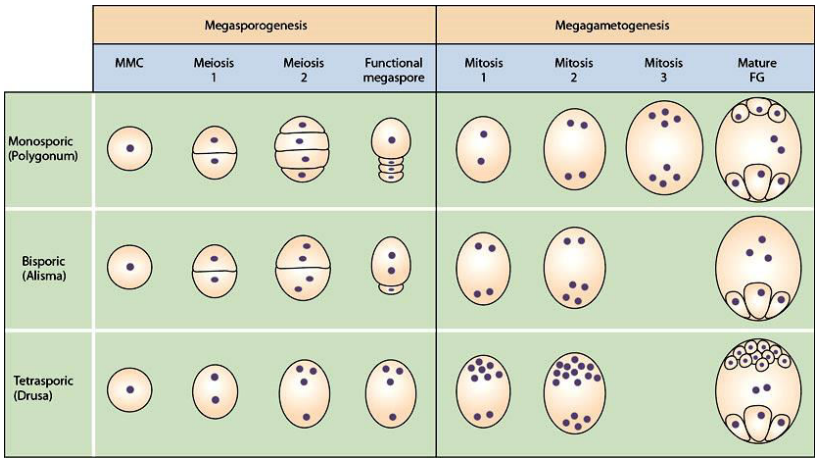
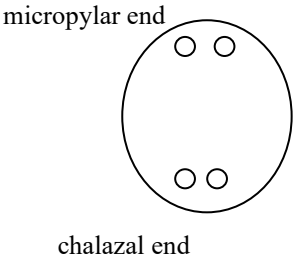


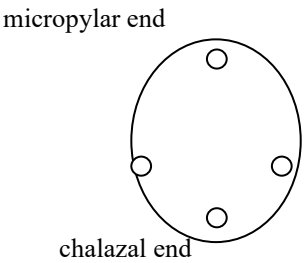
Figure 3.18*-Three main types of embryo sac development: monosporic, bisporic, tetrasporic*

Nuclear behaviour in tetrasporic embryo sac is variable. The arrangement of the four nuclei in the coenomegaspore, before the beginning of post-meiotic mitosis, is of three types.

1. **2+2 arrangement:** two nuclei at the micropylar end and two nuclei at the chalazal end (e.g. Adoxa –type)

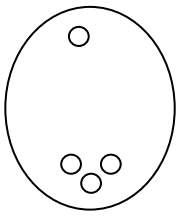


2. **1+1+1+1 arrangement:** one nucleus at the micropylar end, one at the chalazal endand two placed laterally, one on each side. (e.g. Penaea type, Plumbago type, Peperomia type)



3. **1+3 arrangement:** one nucleus at the micropylar end and three at the chalazal end (e.g. Drusa type, Fritillaria type, Plumbagella type)

micropylar end



chalazal end

According to P. Maheshwari (1950), there are 7 types of tetrasporic embryo sacs-

1. Adoxa type
2. Penaea type
3. Plumbago type
4. Peperomia type
5. Drusa type
6. Fritillaria type
7. Plumbagella type

Depending on the following features tetrasporic embryo sacs are of many types:

1. whether nuclear fusion occurs or not,
2. number of the post-meiotic mitoses in the coenomegaspore and
3. final organization of the embryo sac

I-No nuclear fusion occurs

1. Adoxa-type: 2+2 arrangement: two nuclei at the micropylar end and two nuclei at the chalazal end. The embryo sac is eight nucleate, formed after **single post-meiotic mitosis**, having similar organization to that of the Polygonum-type. Examples- In *Adoxa, Sambucus, Ulmus* in the dicotyledons and to certain species of *Erythronium,* and *Tulipa* in monocotyledons.

2. Penaea-type: 1+1+1+1 arrangement**:** one nucleus at the micropylar end, one at the chalazal endand two placed laterally, one on each side. As a result of **two post-meiotic mitosis** in the coenomegaspore, sixteen nucleate embryo sac is formed. The mature embryo sac has 4 groups of 3 cells each, one group is at the micropylar end, one at the chalazal end and two arranged laterally. The remaining four nuclei at the center are polar nuclei. The micropylar triad functions as the egg apparatus.

3. Plumbago-type: 1+1+1+1 arrangement**:** one nucleus at the micropylar end, one at the chalazal end and two placed laterally, one on each side. In this type only **one post-meiotic mitosis** occurs but the organization of mature embryo sac is different from that in Adoxa-type. The mature embryo sac has an egg cell and a four- nucleate central cell. The other three nuclei are cut-off as peripheral cells.

4. **Peperomia type: 1+1+1+1 arrangement:** one nucleus at the micropylar end, one at the chalazal endand two placed laterally, one on each side. Embryo sac is 16 nucleate, like Penaea-type (**two post-meiotic mitosis**) but here an egg apparatus comprising an egg and only one synergid, six peripheral cells and a central cell with eight polar nuclei.

5. Drusa-type: 1+3 arrangement**:** one nucleus at the micropylar end and three at the chalazal end. Embryo sac is also 16 nucleate (**two post-meiotic mitosis**) but the mature embryo sac has a normal egg apparatus (3-celled), two polar nuclei, and 11 antipodal cells.

II-After the second meiotic division three chalazal megaspore nuclei fuse to form a triploid nucleus of the coenomegaspore. The fourth nucleus at the micropylar end remains haploid.

6. Fritillaria-type: 1+3 arrangement**:** one nucleus at the micropylar end and three at the chalazal end.The true sequence of events in the embryo sac development of *Lilium* and *Fritillaria* was fully understood on the basis of investigations of Bambacioni (1928) and Cooper (1935).

In *Lilium* and *Fritillaria* the four megaspore nuclei behave in peculiar and distinctive manner. Three of these nuclei migrate to the chalazal end of the sac and the remaining nucleus is situated at the micropylar end.

Steps:

-The 1+3 arrangement represents the **first**, **four-nucleate stage** in the development of embryo sac.

-The micropylar nucleus divide to form two haploid nuclei, the three chalazal nuclei fuse (triploid) and subsequently divide to form two triploid nuclei. As a result, a **second, four-nucleate stage** is observable, consisting of two haploid micropylar nuclei and two triploid chalazal nuclei.

-A final (fourth) nuclear division occurs, producing four haploid micropylar nuclei (a micropylar quartet) and four triploid chalazal nuclei (a chalazal quartet).

As a result of these events in development, the mature embryo sac of *Fritillaria* or *Lilium* has the following structure: a normal haploid egg apparatus, three triploid antipodal cells and a tetraploid secondary nucleus formed by the union of a haploid and a triploid polar nucleus. When double fertilization occurs the polar nuclei fuse with one of the male gametes and the primary endosperm nucleus is thus pentaploid (5n).

7. Plumbagella-type: 1+3 arrangement**:** one nucleus at the micropylar end and three at the chalazal end. After the nuclear fusion at the chalazal end, both nuclei in the coenomegaspore divide once. As a result four nuclei are formed, two at the micropylar end (haploid) and two at the chalazal end (triploid). One of the haploid nuclei at the micropylar end forms egg and other one functions as the upper polar nucleus. Similarly one of the triploid nuclei at the chalazal end forms a single antipodal cell and the other one functions as the lower polar nucleus. Therefore, just like Fritillaria- type, one polar nucleus is haploid and the other triploid.

Figure 3.19 is showing different types of embryo sacs in angiosperms.

Important Points:

- The 7-celled organization of embryo sac (3-celled egg apparatus, three antipodal cells, and a binucleate central cell) is most common among angiosperms.
- In addition to Polygonum-type, this organization occurs in Allium, Endymion, Adoxa and Fritillaria types of embryo sac.
- -The occurrence of egg cell is universal and it is always haploid.
- -Except for Plumbago and Plumbagella type, the egg cell is always associated with two (rarely one, as in Peperomia type) synergids.
- -The antipodals are always present, except in Oenothera type. Their number and ploidy is, however, variable.
- Monosporic embryo sac: embryo sac develops from one functional megaspore.
- Bi-sporic embryo sac: embryo sac develops from two functional megaspore nuclei.
- Tetra-sporic embryo sac: MMC by meiosis forms four haploid daughter nuclei. No wall formation is there between these four nuclei and all the four megasore nuclei participate in the formation of embryo sac.

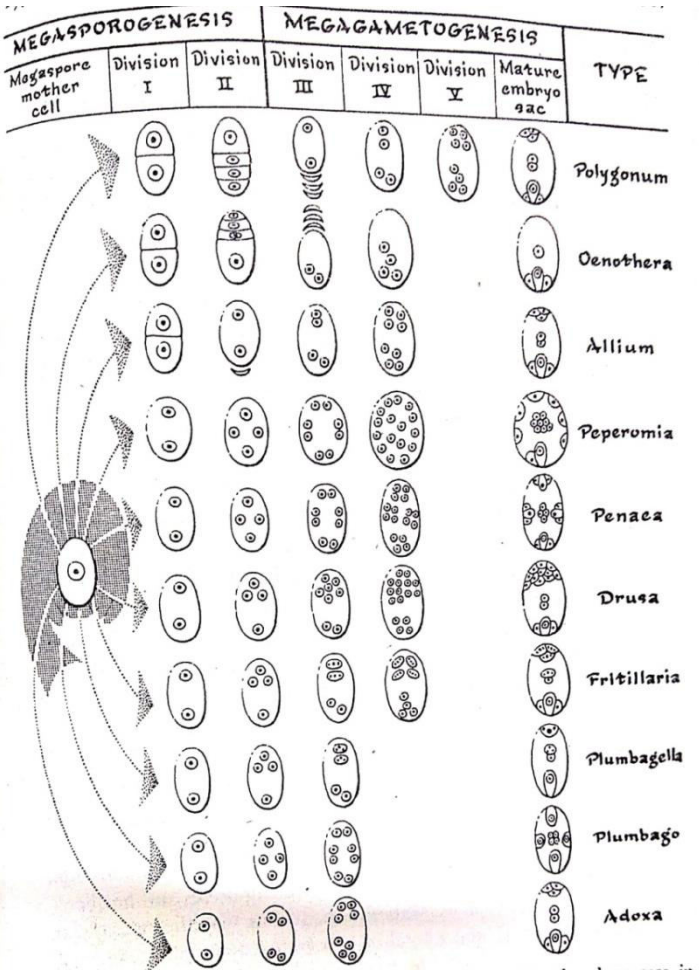


Fig. 3.19-*Types of embryo sacs in angiosperms (Modified from Maheshwari, Bot.Rev.14:1, 1948)*

- The nuclei of the bisporic and tetrasporic embryo sacs are genetically not identical as they are in monosporic embryo sacs, because they arise from two or four different meiotic products.

II-Variation in the formation of meiotically unreduced (diploid) female gametophytes during gametophytic apomixis (Drews and Koltunow 2011)

We know that there are two different forms of apomixis in angiosperms and these are:

1. Sporophytic apomixis

Here somatic cell of an ovule adjacent to a developing embryo sac develops directly into an embryo. So we can say that sporophytic apomixis bypasses female gametophyte formation. Example- in *Citrus* and mango.

2. Gametophytic apomixis

It involves formation of a meiotically unreduced (i.e. diploid) female gametophyte. The diploid egg cell then forms an embryo by parthenogenesis (i.e. without fertilization) and

endosperm formation may be either autonomous (i.e. occurring without fertilization) or pseudogamous (i.e. occurring in response to fertilization of the central cell).

Two forms of gametophytic apomixis have been reported in angiosperms.
1. Diplospory and
2. Apospory
As there are two forms of gametophytic apomixis have been reported in angiosperms, so the formation of a meiotically unreduced (i.e. diploid) female gametophyte is also of two types.

1. Diplosporous female gametophyte development

The megaspore mother cell (MMC) either undergoes an abortive meiosis, as a result of which there is no meiotic reduction and recombination or directly undergoes megagametogenesis. In either case resulting female gametophyte is diploid means unreduced (Figure 3.20). Example- *Arabidopsis* relative few *Boechera* species and some *Tripsacum* species that are relatives of maize.

2. Aposporous female gametophyte development

A megaspore mother cell forms and meiosis starts. Side by side, somatic cells of the ovule, known as aposporous initials (AIs), enlarge near developing megaspore and form unreduced female gametophyte. Few aposporous species,e.g. *Hieracium* subgenus *Pilosella* and *Pennisetum*, shows degeneration of the adjacent sexual megaspores during aposporous female gametophyte formation. In other species, sexual female gametophyte development is not affected and meiotically reduced and unreduced aposporous gametophytes co-exist in the same ovule.

Aaposporous initial cells (diploid) differentiate during megasporogenesis close to sexually programmed cells and undergo mitosis and thus forming a diploid female gametophyte. Time of formation of aposporous initial cells is different in different apomictic species, either soon after formation of megaspore mother cell, during meiotic tetrad development or at the time of selection of functional megaspore. In some species, both haploid and aposporous gametophytes can co-exist in ovules while in others the sexual pathway terminates, usually during early mitotic divisions of the aposporous initial cell (Figure 3.20). Example- *Hieracium* subgenus *Pilosella* species, relative of sunflower and grass genera such as *Pennisetum* and *Brachiaria*.

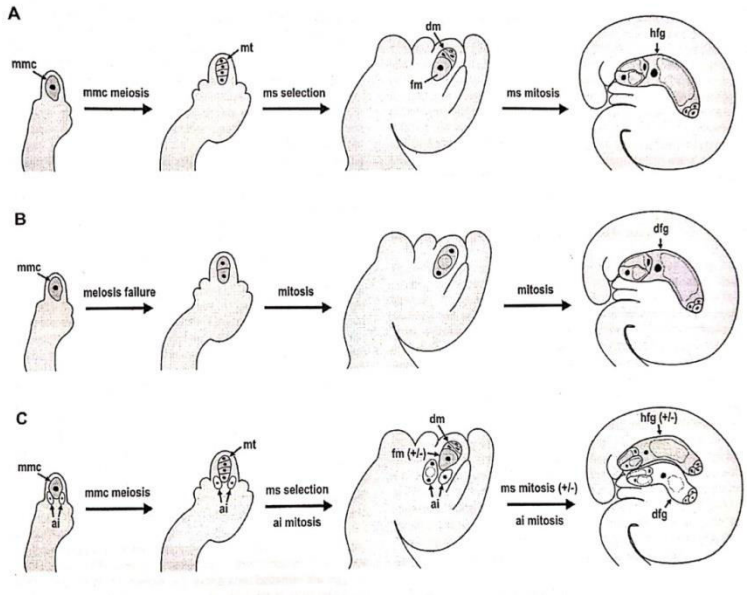


Fig. 3.20 Female gametophyte development in diplosporous and aposporous apomicts compared with Arabidopsis:

(A) Steps of megasporogenesis and megagametogenesis in Arabidopsis ovule
(B) Steps in diplosporous female gametophyte development. The megaspore mother cell enters meiosis and the process fails with the resultant diploid cell undergoing mitosis to form a diploid female gametophyte. Alternatively, the megaspore mother cell may directly undergo mitosis to form the diploid female gametophyte
(C) Steps in aposporous female gametophyte development. Megaspore mother cell differentiation occurs and it can undergo megasporogenesis and megagametogenesis to form a haploid female gametophyte.
ai: aposporous initial cells; dfg: diploid female gametophyte; dm: degenerating megaspores; fm: functional megaspore; hfg: haploid female gametophyte; mmc: megaspore mother cell; mt: meiotic tetrad; (+/-): may be present or absent.

3.6 SUMMARY

In this unit we have discussed the development and structure of ovule, types of ovule on the basis of the position of the micropyle with respect to the funicle, as well as on the basis of dependency on the extent of development of the nucellus and on the basis of position of sporogenous cell. Further development and structure of female gametophyte with special reference to *Arabidopsis* (Polygonum type) was described. The meiotic division of the megasporocyte in the ovule produces a linear series of four haploid megaspores, the lowermost of which most commonly enlarges and by means of three successive nuclear divisions produces an eight-nucleate embryo sac. Types of female gametophyte

development including monosporic, bisporic and tetrasporic type, were also discussed. In addition to these alternative patterns of female gametophyte development were also mentioned. Therefore, the whole unit is summarized in the following key points:

- Gametophyte is the haploid generation producing gametes in plants.
- The female gametophyte (embryo sac) develops within the ovule.
- Ovule consists of nucellus surrounded by integuments.
- Ovule, on the basis of the position of the micropyle with respect to the funiculus, is of 6 types- Orthotropous, Anatropous, Campylotropous, Amphitropous, Hemianatropous and circinotropous.
- Ovule depending on the extent of development of the nucellus and on the basis of position of sporogenous cell, is of 2 types – Tenuinucellate and Crassinucellate.
- Female gametophyte located in the nucellus, develop from megaspore.
- Female gametophyte development occurs in two phases- megasporogenesis and megagametogenesis
- The process of development of the megaspores (n) from megaspore mother cell (2n) by meiotic division is termed as megasporogenesis.
- Out of four megaspores, only one becomes functional.
- The functional megaspore (in normal type) now forms the female gametophyte.
- So megaspore is the first cell (mother cell) of the female gametophyte.
- Development of the female gametophyte is completely endosporous (within the megaspore).
- Female gametophyte which develops from a single megaspore and has eight nuclei is said to be monosporic 8-nucleate embryo sac or Polygonum type (normal type) of embryo sac. It has 3 antipodal cells at chalazal end, an egg apparatus (one egg cell and two synergids) at micropylar end and two polar nuclei in the centre (total 8 nuclei).
- Two polar nuclei later fuse to form secondary nucleus.
- The central cell is binucleate or diploid and the antipodal cells and cells of the egg apparatus are uninucleate and haploid.
- Depending on the basis of involvement of number of megaspore nuclei, the embryo sac can be of 3 types- monosporic, bisporic and tetrasporic.
- The nuclei of the bisporic and tetrasporic embryo sacs are not genetically identical as they arise from two or four different meiotic products.
- The occurrence of egg cell is universal and it is always haploid.
- Except for Plumbago and Plumbagella type, the egg cell is always associated with two (rarely one, as in Peperomia type) synergids.
- The antipodals are always present, except in Oenothera type. Their number and ploidy is, however, variable.
- In angiosperms two forms of gametophytic apomixis have been reported.
- Gametophytic apomixis involves formation of a meiotically unreduced (i.e. diploid) female gametophyte.

- As there are two forms of gametophytic apomixis have been reported in angiosperms, so the formation of a meiotically unreduced (i.e. diploid) female gametophyte is also of two types, i.e., Diplosporous and Aposporous.

3.7 GLOSSARY

Apomixis: a reproductive mechanism that bypasses the sexual process
Bisporic pattern: two-nucleate megaspore (dyad cell) contributes to formation of the mature female gametophyte.
Bitegmic ovule: ovule with two integuments
Chalaza: basal part of the ovule
Crassinucellate ovule: where the sporogenous cell becomes sub-hypodermal, either due to formation of parietal cells, or due to divisions in the nucellar epidermis, or both
Embryo sac: female gametophyte located in the nucellus
Endosporous: within the megaspore.
Filiform apparatus: is highly thickened structure of synergids cell wall at the micropylar end, consisting of numerous finger-like projections
Funicle: A stalk of the ovule by which it remains attached to the placenta
Hilium: region where body of the ovule fuses with funicle
Integument: the protective covering of nucellus
Megagametogenesis: development of the mature female gametophyte from functional megaspore
Megasporocyte: a diploid megaspore mother cell in plants
Megasporogenesis: development of the megaspore from megaspore mother cell
Meiosis: Reduction division occurs in the germ cells.
Micropyle: small opening formed by integument(s)
Mitosis: Non-reductional division of a somatic cell
Monosporic pattern: one megaspore contributes to formation of the mature female gametophyte
Nucellus: Parenchymatous mass of the cells forming main body of ovule
Placenta: the part of the carpellary tissue to which the ovules are attached
Placentation: the arrangement of ovules in the ovary
Synergids: the cells present on either side of egg cell
Tenuinucellate ovule: where the sporogenous cell is hypodermal and the nucellar tissue around it remains single layered
Tetrasporic pattern: four megaspore nuclei take part in the development of female gametophyte.
Unitegmic ovule: ovule with a single integument

3.8 SELF ASSESSMENT QUESTIONS

3.8.1 Multiple choice questions

1. Ovule is also known as

a. megasporangium b. microsporangium

c. embryo sac d. endosperm

2. Stalk by which ovule is attached to the placenta is

a. helium b. funiculus

c. style d. none

3. Another name for female gametophyte is

a. megasporangium b. embryo sac

c. endosperm d. nucellus

4. Orthotropous ovule is also known as

a. hemitropous b. antropous

c. otropous d. atropous

5. Tenuinucellate and crassinucellate are the types of

a. spores b. tetrad

c. ovule d. endosperm

6. Linear tetrad is formed by two meiotic divisions in MMC. Both the divisions are

a. 1^{st} division is transverse, 2^{nd} is vertical b. 1^{st} division is vertical, 2^{nd} is transverse

c. 1^{st} division is transverse, 2^{nd} is also transverse d. may be any type.

7. Which cell in the embryo sac represents the female gamete

a. egg cell b. egg apparatus

c. Synergids d. polar nuclei

8. Female gametophyte is 7-celled, 8-nucleate structure in

a. peperomia type b. polygonum type

c. oenothera type d. none of these

9. Secondary nucleus in the embryo sac is

a. triploid b. haploid

c. diploid d. absent

10. The product of fusion of two polar nuclei is

a. zygote b. oosphere

c. embryo sac d. secondary nucleus

11. Which one of the following is the example of monosporic type of embryo sac
a. polygonum type b. allium type
c. endymion type d. peperomia type

12. Which one of the following is the example of bisporic type of embryo sac
a. plygonum type b. allium type
c. oenothera type d. peperomia type

13. Which one of the following is the example of tetrasporic type of embryo sac
a. polygonum type b. allium type
c. endymion type d. peperomia type

14. Megasporogenesis is a process in which
a. microspores are formed b. megaspores are formed
c. both of the above d. none of the above

15. Ovules in future convert into
a. fruits b. leaves
c. seeds d. roots

16. Primary endosperm nucleus is pentaploid in
a. *Oenothera* b. *Polygonum*
c. *Peperomia* d. *Fritillaria*

17. Oenothera type of embryo sac is derived from the
a. chalazal megaspore of the tetrad b. micropylar megaspore of the tetrad
c. any of the megaspore of the tetrad d. none of the above

Answer key 3. 8.1:
1.(a); 2.(b) 3.(b); 4.(d); 5.(c); 6.(c); 7.(a); 8.(b); 9.(c); 10.(d) 11.(a); 12.(b); 13.(d); 14.(b); 15.(c); 16.(d); 17.(b).

3.8.2 Short answer type questions

1. Name the 1st cell of female gametophyte.
2. Name the last cell of female sporophyte.
3. What is endosporous type of development?
4. What do you understand by tenuinucellate type and crassinucellate type of ovule?
5. What is megasporocyte?
6. What is megasporogenesis?
7. How many types of ovule are there? On what basis they are classified?

8. What is the main criterion for classifying embryo sac?
9. Why the 7-celled embryo sac is called Polygonum type?
10. Differentiate the bi and tetrasporic embryo sacs.

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3.11 TERMINAL QUESTIONS

1. Describe the different types of ovule.
2. Explain the patterns of megasporogenesis and megagametogenesis in angiosperms.
3. Compare monosporic embryo sac with bisporic and tetrasporic embryo sac.
4. Give a detail account of the development of ovule, megaspores and embryo sac.
5. With the help of labelled diagram give the development of female gametophyte in *Polygonum.*
6. Describe various types of ovules found in angiosperm with suitable diagrams.
7. Describe aposporous and diplosporous female gametophyte development and compare it with development in Arabidopsis.

UNIT-4: DOUBLE FERTILIZATION, ENDOSPERM AND EMBRYO DEVELOPMENT

4.1OBJECTIVES

Students will be able to answer the following questions:

- What is Fertilization?
- What are the different ways of entry of pollen tube into the ovule.
- What is syngamy?
- What do you understand by triple fusion?
- In angiosperms double fertilization is a unique phenomenon. Students will clearly understand about double fertilization.
- What is the fate of products of triple fusion and syngamy?
- What is endosperm? How it forms and on what basis endosperm is categorized into different types?
- What is an embryo?
- While going through the chapter, students will encounter the development of dicotyledonous and monocotyledonous embryo.
- Students will also become familiar about the above said embryo development with different examples.

4.2 INTRODUCTION

After going through the male gametophyte and female gametophyte units, it is now clear that gametophytic generation is haploid. The first male gametophytic cell is microspore and the first female gametophytic cell is megaspore. The pollen grains are liberated at the 2-celled or 3-celled stage. Female gametophyte is also known as embryo sac and in majority of the species it is of Polygonum type. After development of male and female gametophytes (Fig. 4.1) the next biological phase is pollination, which is must for fertilization. Pollination ends in a copious dusting of the stigma surface with pollen grains.

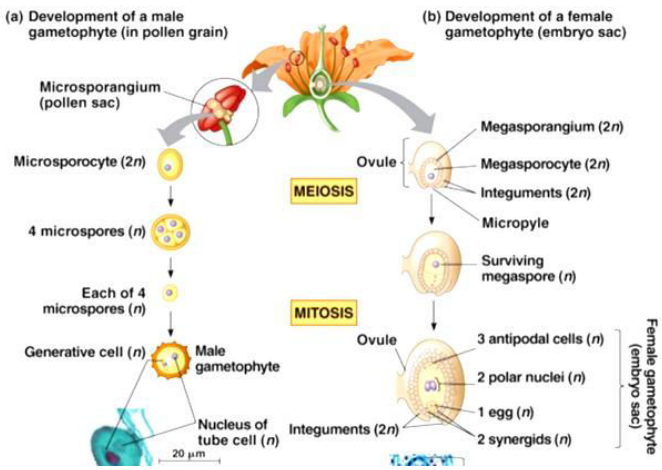


Fig. 4.1- *Development of male and female gametophytes*

In this unit we will discuss about fertilization and post fertilization developments. It will include endosperm development as well as embryo development with the help of specific examples. Especially embryo development will be discussed by taking important examples in both dicot and monocot plants in detail.

The capacity to reproduce is one of the most important characteristics of life and is aimed to sustain the individual species. Sexual method of reproduction requires fusion of two gametes, one from male organ and other from female organ of the plant. The product of the fusion of two different gametes is zygote and this fusion process is known as fertilization.

In angiosperms fertilization initiates with the compatible pollens reaching the stigma and ends with the fusion of male and female gametes in the embryo sac. The pollens received by the female reproductive organ i.e., carpel are held at the stigma.

There is no such way by which the pollens having male gametes can reach to the egg (female gamete) in the embryo sac. So to overcome this difficulty pollens germinate on the stigma and forms pollen tube which penetrates the stigmatic tissue, grows down the style, enters the ovary and finally finds its way into the embryo sac (female gametophyte) through ovule. Here it releases two male gametes in the vicinity of the female gametes. Out of the two sperms, one fuses with the egg (syngamy) and forms zygote. The other one fuses with the polar nuclei or the secondary nucleus (triple fusion) and forms primary endosperm nucleus. As the fusion inside the embryo sac taking place twice hence this phenomenon is known as double fertilization and is a characteristic unique feature of the angiosperms.

After a series of divisions, primary endosperm nucleus forms endosperm. Endosperm is a nutritive tissue that nourishes the developing embryo. Zygote or oospore forms either dicotyledonous or monocotyledonous embryo, as the case may be.

Terms to remember:

Apical cell = terminal cell = (also known as embryo cell)

Basal cell = also known as suspensor cell

Meiosis = reduction division

Syngamy = fertilization

Sperms = male gamete

4.3 PRE-FERTILIZATION DEVELOPMENTS:

"Fertilization is the process of fusion of two dissimilar reproductive units, called gametes."

In flowering plants, the process of fertilization was first discovered by Strasburger in 1884. As described in previous unit, the female gametophyte (embryo sac) of angiosperms is situated in the ovule. Therefore the pollens after reaching to the stigma produce a pollen tube which facilitates transport of male gametes deep into the embryo sac from stigma.

The growing pollen tube penetrates the stigmatic tissue and pushes its way through the style and then down the wall of the ovary. The style may be hollow or solid. If it is hollow, then the pollen tube grows along the epidermal surface but in case of solid style, the pollen tube travels through intercellular spaces between the cells which lie in its path. Growth of pollen tube can be seen in longitudinal section of a flower (Fig. 4.2).

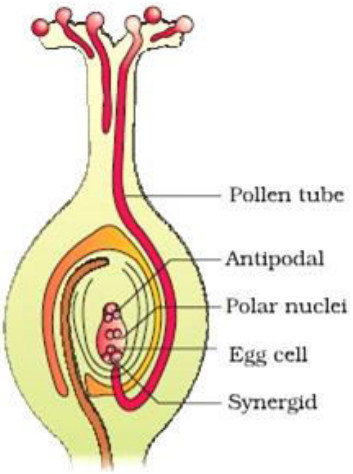


Fig. 4.2- *Longitudinal section of a flower showing growth of pollen tube*

Entry of pollen tube into ovule:

After arriving in the ovary, the pollen tube finds its way into the ovule. The pollen tube may enter into the ovule via three routes (Figure 4.3).

1. through the micropyle
2. through the chalazal end
3. through the integument or through the funicle

On that basis of modes of entry of pollen tube into the ovule, three terms are given as follows:

1. Porogamy:

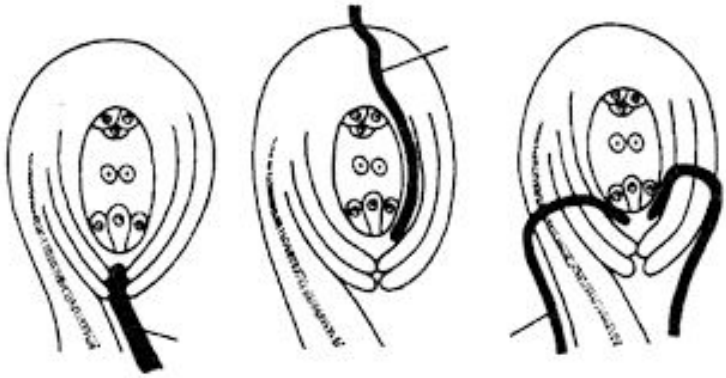
When the pollen tube enters the ovule through the micropyle, the condition is known as **porogamy**. This is the most common mode of pollen tube entry into the ovule.

2. Chalazogamy:

When the pollen tube enters the ovule through the chalazal end, the condition is known as **chalazogamy**. This type of pollen tube entry into the ovule was observed in *Casuarina, Betula* and *Juglans regia*. The chalagogamy was first reported by Treub (1891) in *Casuarina*.

3. Mesogamy:

When the pollen tube enters the ovule through the integument or through the funicle, the condition is known as **mesogamy**. This type of pollen tube entry into the ovule was observed in *Cucurbita* (through the integument), and *Pistacia* (through the funicle).



Porogamy Chalazogamy Mesogamy

Fig. 4.3 *Modes of entry of pollen tube into the ovule*

Entry of pollen tube into the embryo sac:

It does not matter through which way pollen tube enters into the ovule; it always enters in the embryo sac through the micropylar end means **entry of pollen tube in the embryo sac is irrespective of pollen tube entry into the ovule**.

Again the entry of pollen tube into the embryo sac after passing micropyle may be via different passages. It may be:

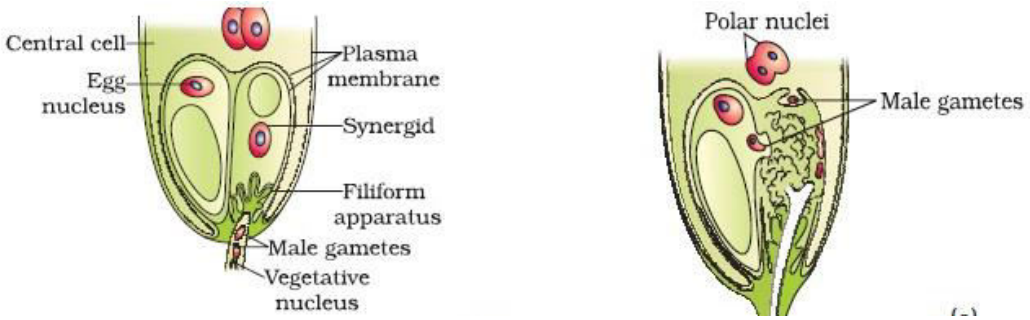
(i) between the egg cell and one of the synergids,
(ii) between the wall of the embryo sac and synergid
(iii) directly penetrating one of the synergids.

So we can say that synergids not only play an important role in determining the entry of pollen tube in the embryo sac but they also affect dissemination of male gametes in the embryo sac.

Discharge of male gametes from pollen tube:

After reaching the embryo sac the tip of the pollen tube swells up and finally ruptures liberating two male gametes into the cytoplasm of the female gametophyte. Just prior to bursting of pollen tube the tube nucleus disorganizes. Immediately after release, the male gametes show amoeboid movement and one male gamete moves toward the egg while the other one moves towards the polar nuclei (or secondary nucleus). By going through Fig. 4.4

you could see the path of pollen tube and release and movement of male gametes inside the female gametophyte.



A **B**

Fig. 4.4- A*: enlarge view of an egg apparatus showing path of pollen tube growth*
B*: discharge of male gametes into a synergid and the movement of the male gametes, one into the egg and the other into the central cell*

Syngamy- fusion of gametes:

When one of the male gamete reaches the egg, it fuses with it. As a result of this fusion diploid zygote/ oospore (2n) forms. The fusion of male and female gametes is known as **syngamy** or **fertilization** (Fig. 4.5).

This is one of the most significant discoveries made by **E. Strasburger in 1884**. He observed the actual fusion of the male gamete with the female gamete (egg) in *Monotropa.*

Since two male gametes are released by the pollen tube, what happened to the second male gamete? The answer was provided by **S. Nawaschin (1898)** while working with *Fritillaria* and *Lilium.* He showed that the one male gamete fuses with the egg (syngamy) and the other male gamete fuses with the two polar nuclei forming a triploid nucleus (3n) known as **primary endosperm nucleus** (Fig. 4.5).

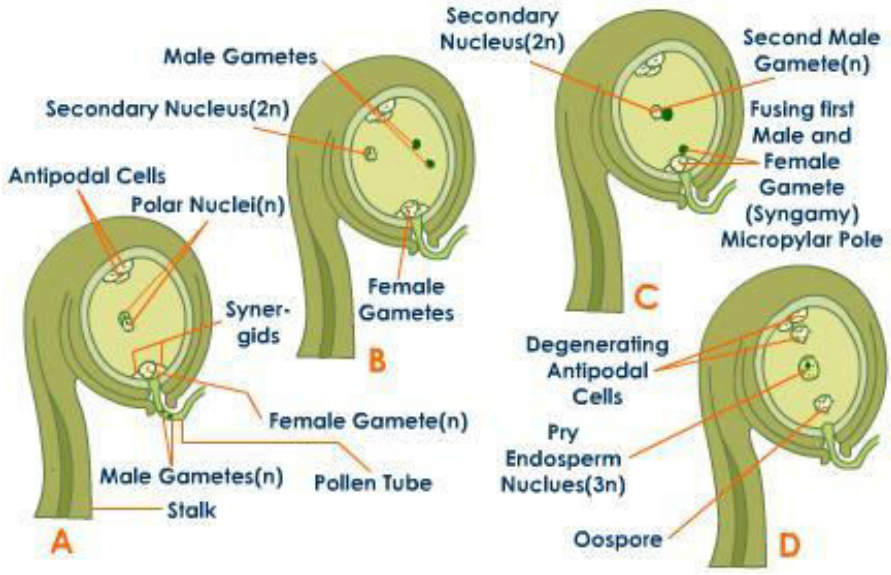


Fig. 4.5*- Syngamy and triple fusion*

4.4 DOUBLE FERTILIZATION

In an embryo sac fusion occurs twice, one is **syngamy** and another is **triple fusion** and therefore, the phenomenon is known as **double fertilization**.

As a result of first fusion, the zygote or **oospore** cell is formed which is the **first cell of the sporophyte (embryo)**. The nucleus of the triple fusion product is triploid or 3n and known as **primary endosperm nucleus** (Fig. 4.5) and it gives rise to the endosperm.

> **Double fertilization** is a very unique phenomenon in angiosperms and **discovered for the first time by S.G. Nawaschin (1898) in *Lilium* and *Fritillaria* species**.

Post Fertilization Developments

After fertilization, development of the **embryo** and the **endosperm** within the embryo sac goes side by side. The **oospore (zygote), develops into the embryo** while the **primary endosperm nucleus develops the endosperm**. The other nuclei or cells within the embryo sac (synergids, antipodal cells) disorganize sooner or later.

Since the primary endosperm nucleus is the result of triple fusion, this is characterized by both maternal and paternal chromosomes or in other words developing endosperm has hybrid vigour.

There are some angiosperms where endosperm formation is suppressed or you can say that they do not form endosperm. Such angiosperms are the members of Orchidaceae, Podostemaceae and Trapaceae.

[If we are talking about **the importance of endosperm for human beings**, it forms the edible part of cereals and coconut and is a source of commercial castor-oil in castor-bean. Major part of human calories comes from endosperm, which means endosperm is considered the most important plant product on earth for man.

Cereals like wheat, rice maize, millets, barley and oats and their diverse commercial products like cornflakes, popcorn, bear etc. are endosperm. Similarly cooking oil i.e., coconut oil, corn oil and industrial oil i.e., palm oil, castor bean oil, Jatropha oil are of endosperm origin.**]**

Functionally endosperm is comparable to the female gametophyte in gymnosperms but has a unique origin. In gymnosperms endosperm differentiates before fertilization and is haploid whereas in angiosperms mostly it is a product of triple fusion and is triploid.

4.5 ENDOSPERM DEVELOPMENT

Endosperm is a tissue which provides the essential food materials utilized in the growth of the embryo and in many cases, the young seedling. So endosperm, a fundamental component in the evolutionary success of angiosperms (Stebbins 1974), is not only the important source of food for the developing embryo but also an important source of food, feed and industrial raw materials for mankind.

As describe earlier, the primary endosperm nucleus is formed either by the fusion of one haploid male gamete and one diploid secondary nucleus (fusion product of two haploid polar nuclei) or by the fusion of three haploid nuclei (one male gamete belongs to male gametophyte and two polar nuclei belong to the female gametophyte).

[**Exceptions are there**, when we are talking about triple fusion in angiosperms. In Onagraceae, there is only single polar nucleus, and hence the expression “triple fusion” is meaningless (Fig. 4.6, *Oenothera*). In certain other genera four, eight or as many as fourteen polar nuclei join with one of the male gamete in the formation of primary endosperm nucleus (Figure 4.6). So there is wide variation in the number of polar nuclei which join with the nucleus of male gamete in the initiation of endosperm.

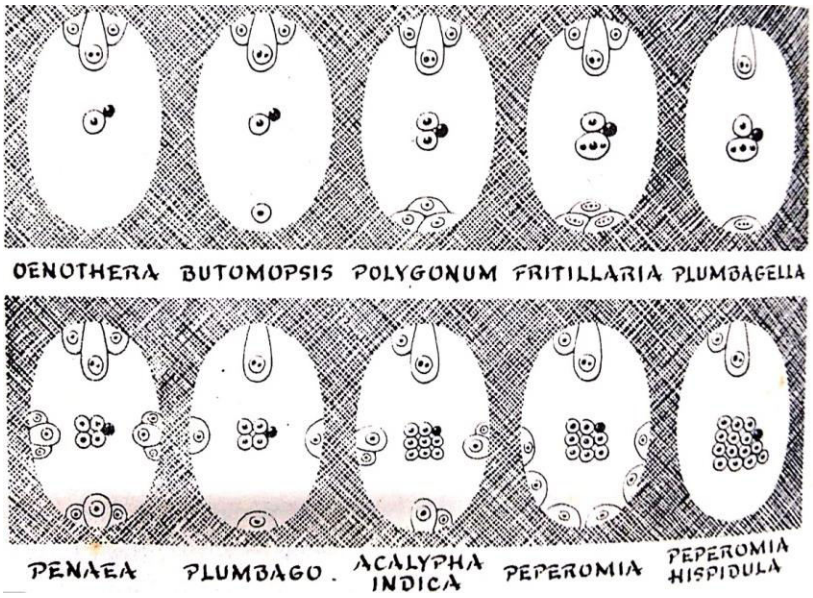


Figure 4.6- *Embryo sacs. Wide variation in the number of polar nuclei which join with the sperm nucleus (shown in black) in the initiation of endosperm. [Adopted from An introduction to the Embryology of Angiosperms by P.Maheshwari. N.Y. McGraw Hill Book Company, Inc., 1950]*

Generally the endosperm nucleus divides after the division of the oospore, but in many cases the endosperm is formed even before the first division of the oospore.

In triple fusion process there is fusion of only the male nucleus with the polar nuclei, male cytoplasm does not take part in the process while the membrane of the primary endosperm nucleus is formed by both the secondary nucleus and the male nucleus.

After fertilization, metabolic activity increase in the central cell and protein –synthesis machinery organized for the differentiation of primary endosperm cell (Bhatnagar and Sawhney 1980).

Depending upon mode of development three types of endosperm has been recognized:

1. nuclear endosperm
2. cellular endosperm
3. helobial endosperm

Out of these nuclear endosperm is the most common type which occurs in about 56% families of angiosperms. It is followed by cellular endosperm (reported in 25% families of angiosperms) and then by helobial endosperm (reported in 19% families of angiosperms).

1. Nuclear endosperm:

In this type of endosperm the division of primary endosperm nucleus and number of subsequent nuclear divisions are not accompanied by wall formation (free nuclear) and the nuclei thus produced (few to several thousand) remain free in the cytoplasm of the embryo sac. Later nuclei lie peripherally in the cytoplasm due to the appearance of central vacuole. Fig. 4.7 is showing simple way to understand nuclear endosperm formation.

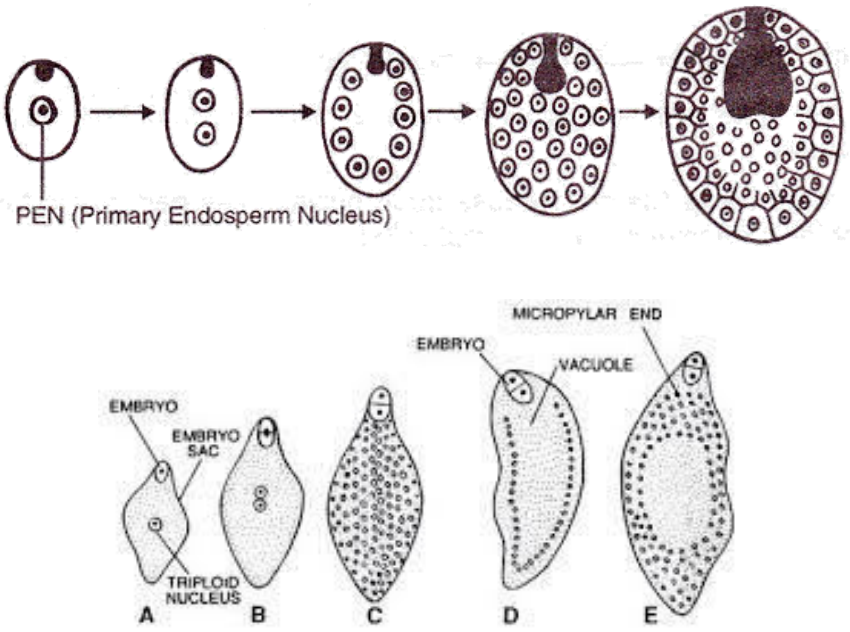


Fig.-4.7 *Simple way to show the formation of Nuclear endosperm*

In nuclear type of endosperm there may be variations:

(1) Free nuclear condition of endosperm may persists until it is consumed by the developing embryo as observed in *Floerkea, Limnanthes, Oxyspora*.

(2) Endosperm may become cellular later on. Wall formation around nuclei occurs at later stage resulting into definable cellular tissue, at least in certain parts of the sac. The wall formation is mostly centripetal, i.e., from the periphery towards the center and usually begins from the basal periphery e.g. *Arachis hypogea.*

Again the degree of cellularization varies. Mostly the endosperm becomes completely cellular e.g. *Acalypha indica* (Fig. 4.8) or cellularization occurs only around the embryo e.g. *Phaseolus* or the wall formation is only in the upper region of the embryo sac.

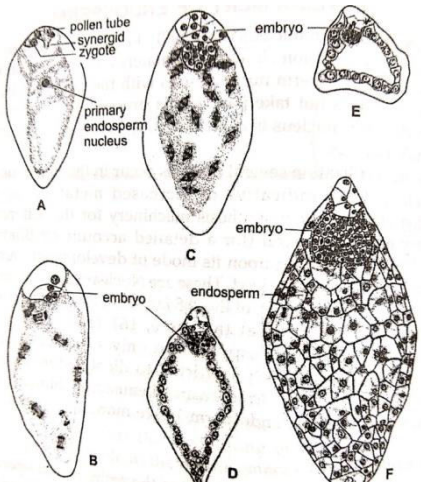
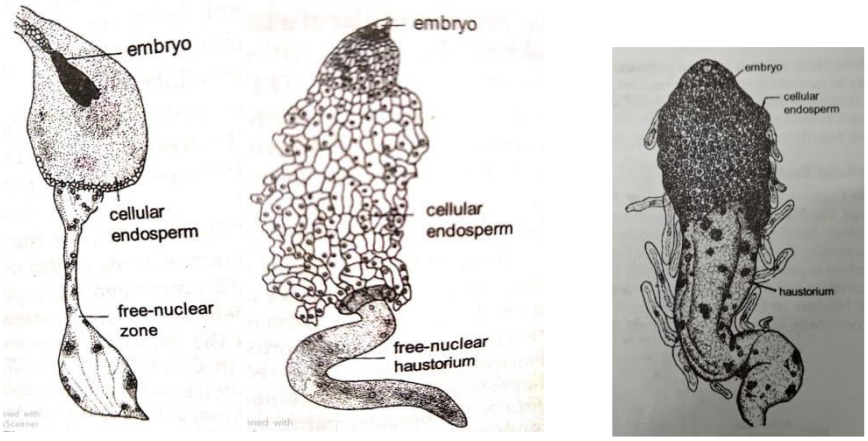


Fig. 4.8 *Nuclear endosperm in Acalypha indica.*

A*.Embryo sac after fertilization; the PEN and the zygote have not yet divided.* ***B, C****.Embryo sac, showing synchronous divisions of the endosperm nuclei.* ***D****.The endosperm nuclei have moved to the periphery.* ***E****.The peripheral part of the embryo sac has become cellular.* ***F****.Completely cellular endosperm. [After Johri and Kapil, 1953]*

Haustorial structures have been observed in many cases when the chalazal region remains free nuclear and elongates. This behaves like a haustorium e.g. *Crotalaria, Grevillea robusta,* several members of Cucurbitaceae, Fabaceae, and Proteaceae. In addition to the main chalazal haustorium, many single celled, finger like projections are present all over the endosperm of *Lomatia polymorpha* which increases the absorbing surface of the endosperm (Fig. 4.9).



A B C

Fig. 4.9 A. *In Crotalaria the upper-half of the endosperm becomes cellular whereas the lower-half remains free-nuclear (after Rau, 1951).* ***B.*** *In Grevillea robusta the free-nuclear, vermiform appendage (haustorium) at the chalazal end of the cellular part of the endosperm (after Kausik, 1938) and* ***C.*** *In Lomatia polymorpha several uninucleate projections are present on the surface of the endosperm as well as the haustorium (after Venkata Rao, 1963)*

The **longest endosperm haustorium** is reported in *Echinocystis lobata* of the Cucurbitaceae (16 mm in length; Chopra and Seth, 1977).

In some cases the central vacuole formed after free nuclear division, may not be filled up even in the mature seed. This is seen in the palms. ***Cocus nucifera*** **is the classical example of this type of nuclear endosperm.** Development of endosperm in it deserves special mention. The primary endosperm nucleus undergoes a number of free nuclear divisions. Then the embryo sac gets filled with a clear fluid (watery liquid endosperm) in which numerous nuclei float. It is known as **liquid syncytium.** At that time the fruit size is about 50 mm long. Gradually nuclei start settling at the periphery with the beginning of peripheral cell wall formation. This forms the coconut meat (fruit size is about 100 mm long). In mature coconuts the liquid endosperm becomes milky and it does not contain free nuclei. The watery endosperm of coconut contains growth promoting 'coconut milk factor' and that is why it is used as a nutrient medium in plant tissue culture experiments. Nuclear endosperm commonly occurs in polypetalous dicotyledons, maize, wheat, rice, sunflower etc.

Development of endosperm in *Areca catechu* is more or less similar to that in coconut but here the endosperm cavity is smaller and it becomes completely filled by the growth of the endosperm and later on becomes extremely hard.

2. Cellular endosperm:

In this type of endosperm, first division of the primary endosperm nucleus and subsequent other divisions are followed by wall formation so the endosperm is cellular from the beginning. The first wall is laid down transversely but the subsequent divisions are irregular (Fig. 4.10). Example- *Adoxa, Peperomia, Villarsia* etc. occurs commonly in gamopetalous plants.

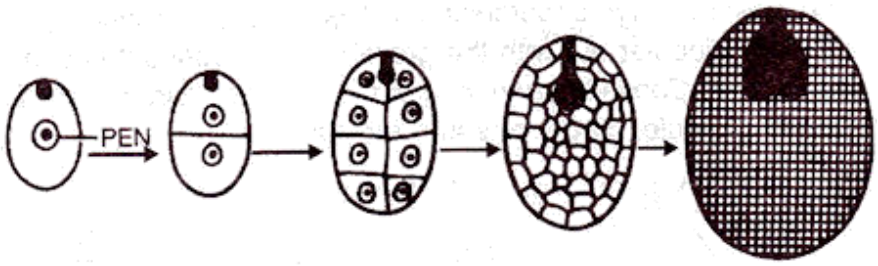


Fig. 4.10: *Simple way to understand cellular endosperm formation*

Both micropylar and chalazal haustoria may also be seen in cellular type of endosperm. Micropylar haustoria are reported in *Impatiens roylei* and *Hydrocera triflora.* Chalazal haustorium is reported in *Magnolia obovata* (Fig. 4.11).

Endosperm development in *Magnolia obovata* (Fig. 4.11)**-** As shown in figure wall formation after first nuclear division is transverse. As a result of which almost two equal size chambers are formed. One is micropylar and another is chalazal. Divisions in micropylar chamber are more rapid and in all directions. In chalazal chamber divisions are transverse and slow in rate. These kinds of divisions in both the chambers produce a tail like chalazal part attached to the more massive tissue at the micropylar end. Further divisions takes place in the upper part of the tail and add to the endosperm tissue. The basal two or three cells of the tail elongate to form a haustorium which penetrates the chalazal part of the nucellus.

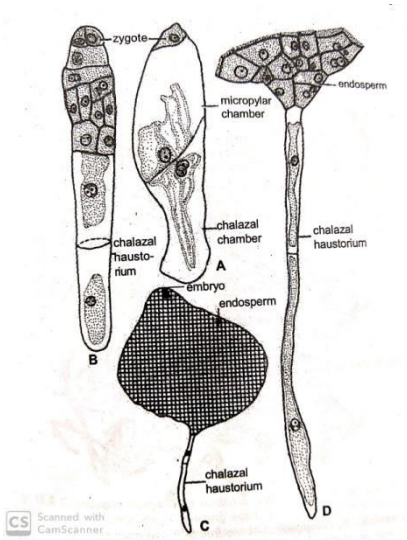


Fig. 4.11: *Endosperm development in Magnolia obovata.* ***A****. Two-celled endosperm.* ***B****. Thirteen –celled endosperm.* ***C****. Endosperm at the globular stage of embryo to show 2-celled chalazal haustorium.* ***D****.A portion from C enlarged to show the chalazal haustorium with a few cells of the endosperm proper (after Kapil and Bhandari, 1964)*

In ***Iodina rhombifolia*** a very aggressive chalazal haustorium is formed (Bhatnagar and Sabharwal 1969). In this plant an extensive caecum is formed in unfertilized embryo sac, the lower end of which extends into the placenta and branches. After first nuclear division in primary endosperm nucleus, micropylar and chalazal chambers are formed by transverse wall formation. The chalazal nucleus migrates into the caecum and becomes hypertrophied (about 600 µM). Profuse branching at the free end gives the haustorium a coralloid appearance (Fig. 4.12).

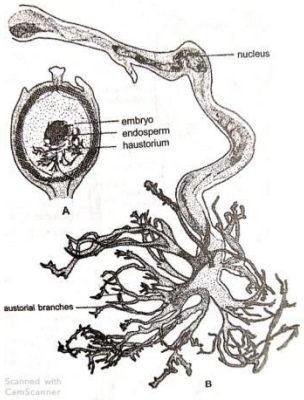


Fig. 4.12- *Iodina rhombifolia. A. Longitudinal section of the ovule at the globular stage of the embryo, note the aggressive nature of the chalazal haustorium. B.Enlarged view of the haustorium with branched lower end and hypertrophied nucleus (after Bhatnagar and Sabharwal, 1969)*

Endosperm development in **Loranthaceae is unique** in the sense that here true ovules are absent and all the embryo sacs in an ovary lie close to each other. After fertilization, the primary endosperm nucleus moves to the basal part of the embryo sac and divides. Endosperms of all the embryo sacs in an ovary fuse to form a **composite endosperm** (Fig. 4.13).

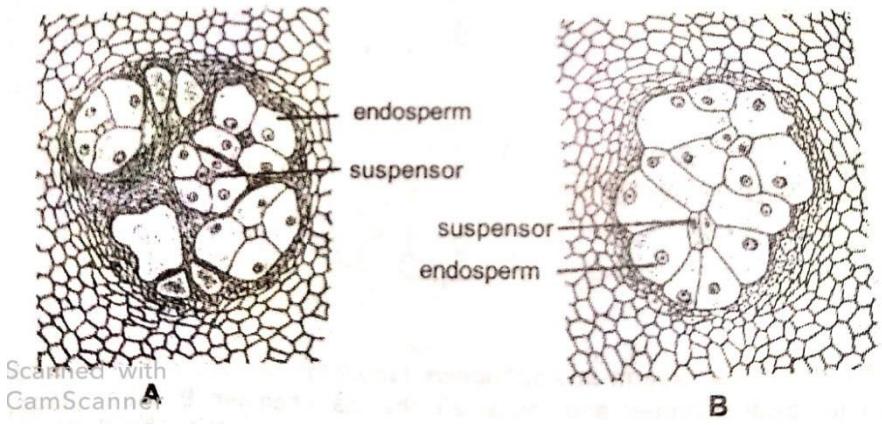


Fig. 4.13- *Composite endosperm in Tolypanthus involucratus. A.Transverse section of ovary, showing four embryo sacs, each with 4-seriate endosperm and a biseriate suspensor. B.Same, at a later stage of development. All the endosperms in the ovary has fused and formed a composite structure (after Dixit, 1961)*

3. Helobial endosperm:

This type, so named because of its occurrence in the members of order Helobiales (monocotyledons), is characteristics of a widely scattered series of angiosperm genera. In

this type of endosperm, the development is intermediate between the nuclear and the cellular types. Here the first division of the primary endosperm nucleus is accompanied by the formation of transverse wall. This divides the embryo sac unequally into two compartments- a small chalazal chamber and a large micropylar chamber (Fig. 4.14)

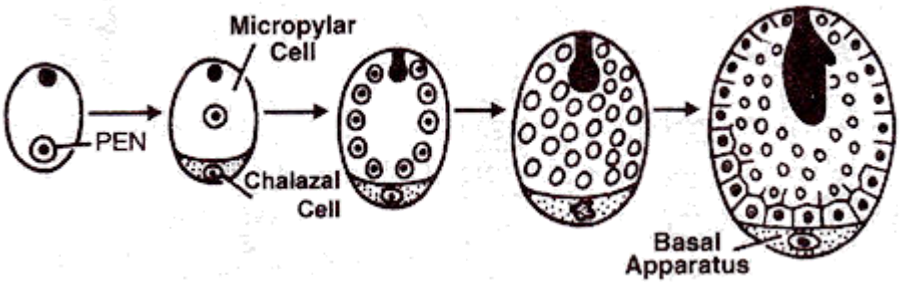


Fig. 4.14- *Simple way to understand Helobial endosperm formation*

Formation of unequal chambers is followed by free nuclear division in both the chambers but there are relatively more free nuclear divisions in micropylar chamber in comparison to chalzal one. The chalazal chamber often degenerates. The free nuclear divisions in the micropylar chamber are followed by wall formation and thus a cellular endosperm tissue is formed. Fig. 4.15 is showing development of the Helobial type of endosperm in *Eremurus himalaicus*.

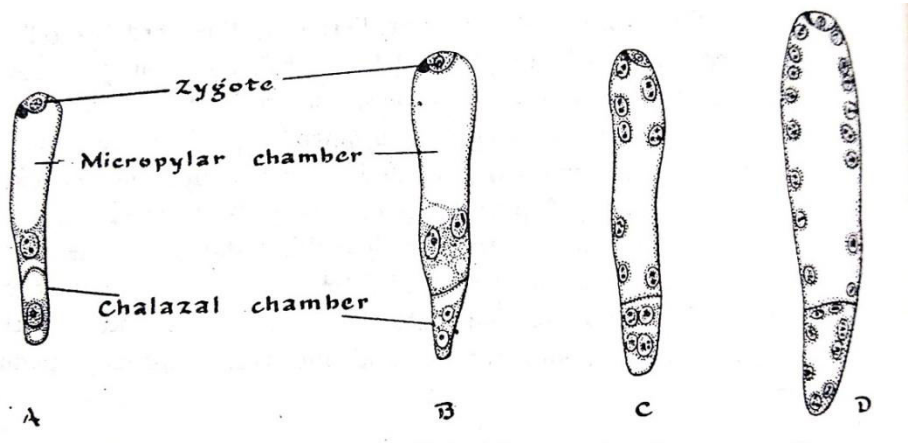


Fig.4.15: *Development of the Helobial type of endosperm in Eremurus himalaicus [Adapted from An introduction to the Embryology of Angiosperms by P.Maheshwari. N.Y. McGraw Hill Book Company, Inc., 1950]*

Endosperm in Cereals

In cereals the endosperm is of the free nuclear type. On maturity it becomes starchy and dry but is few grasses it remains liquid or soft even after attaining maturity (Terrell 1971). According to Becraft *et al.* (2001) endosperm of cereals consists of four distinct types of cells (Fig. 4.16) or tissues-

1. Transfer Cells (TCs),
2. Embryo Surrounding Cells (ESCs),
3. Starchy Endosperm Cells (SECs) and
4. Aleurone cells (ALCs).

Transfer Cells (TCs),

These cells are also known as modified aleurone cells which are located at the chalazal end of the endosperm (in maize) or over the nucellar projection (in barley and wheat). These cells have extensive cell wall ingrowths as a result the plasma membrane surface increases. These invaginations ultimately facilitate nutrient (mainly sucrose and amino acids) uptake by the endosperm.

Embryo Surrounding Cells (ESCs),

There are several layers of cells lining the endosperm cavity in maize. These are known as embryo surrounding cells (ESCs) so completely surrounds the young embryo. ESCs shrink with the growth of endosperm and at maturity only vestigial remnants of these ESCs are seen. Metabolically ESCs are very active and involved in supplying the embryo with sugar. They also protect the embryo from pathogens.

Starchy Endosperm Cells (SECs)

A major site of starch and protein storage is the inner mass of endosperm, consists of starchy endosperm cells (SECs). In mature grains the SECs are dead.

Aleurone cells (ALCs)

Aleurone cells form a sheet. Number of sheets varies in various species like one layer in maize, wheat, and three layers in barley or several layers in rice. Aleurone is the only live tissue of endosperm at maturity.

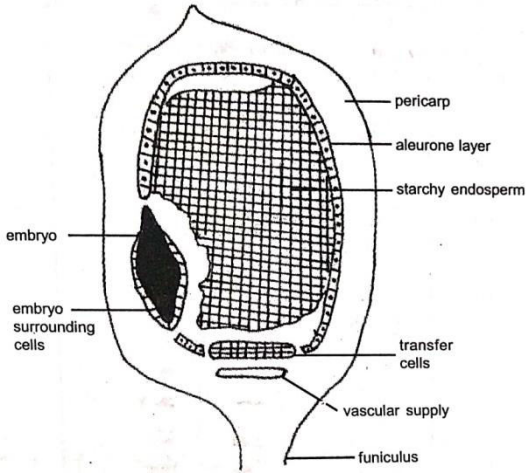


Fig. 4.16- *In an immature grain of maize (10 days after pollination) the starchy endosperm is surrounded by a single layer of aleurone cells and 1-3 layers of transfer cells at the chalazal end over the funiculus. The embryo is partly surrounded by the cells of the embryo surrounding region of the endosperm (after Becraft et al., 2001)*

Importance of aleurone layer

Biological importance

During seed germination when seed imbibes, gibberellic acid from embryo stimulates aleurone cells. In response to stimulation, aleurone cells activate a gene expression programme, as a result of which a range of proteases and amylases synthesize which cause-digestion of endosperm cell wall and breakdown of the stored starch and proteins in the dead starchy endosperm. This whole process makes free sugars and amino acids available to the germinating embryo (Fath *et al*. 2000).

- The aleurone cells are also major sites of mineral storage (Stewart *et al*.1988).
- Protect the nutrient rich central starchy endosperm.
- Controls seed dormancy in case of *Arabidopsis.*

Practical importance

- Almost half of the cereal bran is composed of aleurone cells, which is the most dietarily beneficial fraction of the bran.

Ruminate endosperm

If mature endosperm shows any degree of irregularity and unevenness in its surface, then it is called ruminate endosperm (Fig. 4.17 & 4.18) or we can say that Ruminate endosperm is characterized by its uneven and enlarged surface. Ruminate endosperm is known to occur in about 58 families of angiosperms.

Rumination may be because of:

- the activity of seed coat or
- the endosperm itself

By the activity of seed coat: The irregularities on the inner surface of the seed coat may arise by

1) unequal radial elongation of any one or the only layer of the seed coat (*Passiflora calcarata*) or
2) definite ingrowth or infolding of the seed coat (in Annonaceae (the custard apple family) and Aristolochiaceae)

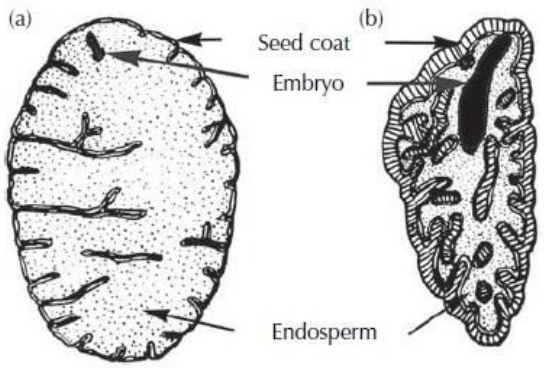


Fig. 4.17- *Ruminate endosperm*

By the endosperm itself:

1) Endosperm begins to increase in volume with the increase in volume of the seed. Soon this endosperm absorbs the nucellus and comes in direct contact with the seed coat. During further growth of the endosperm, the irregular inner surface of the seed coat makes it ruminate. Example- in *Myristica, Coccoloba*

2) The endosperm exhibits unequal peripheral activity during late stage of its development and causes the seed coat to attain an irregular configuration. Example- in *Andrographis, Elytraria*

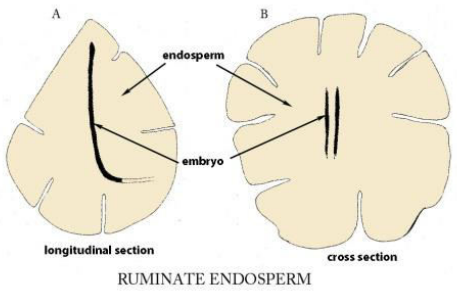


Fig. 4.18 *Ruminate endosperm*

Reserve food substances in Endosperm

Persistent endosperm in mature seeds is rich in reserve food that consists of carbohydrates, fats and/or proteins. This reserve food material of endosperm is digested and utilized by the germinating seed until the seedling develops chlorophyll for photosynthesis. That is why endosperm serves as nutritive tissue not only for developing embryo but also for the growth of the seedlings.

Carbohydrates: In most seeds, the principal storage carbohydrate is starch composed of two α–glucan polymers, amylase and amylopectin. In *Allium cepa, Phoenix dactylifera, Washingtonia filifera* reserve carbohydrates are mannose and xylocans. In Ivory nut (*Phytelephas macrocarpa*) major storage carbohydrate is mannose.

Proteins: Storage proteins globulin and certain albumins occur in all species and prolamines are unique to the cereals. Prolamines (having glutamine and proline) account for 50-60% of the total proteins in cereal endosperm.

There are two basic types of prolamine:

1. Those found in wheat and its relatives like barley and rye. These are gliadins and glutenins.
2. Those found in maize and its relatives like millets and coix. These are zeins.

Lipids: Triacylglycerols in the form of oil are present in endosperm. As- Castor bean, Oil palm, opium poppy and spurge store oil up to 50% of the seed's dry weight.

Role of genetic engineering in improving the nutritive value of endosperm

As we know that cereal endosperm is the major staple food of about one third of the world's population and is a good source of calories but very poor in vitamins and proteins so techniques of genetic engineering have been used to develop the nutritive value of endosperm.

Example- 1.Genetically engineered rice has been developed which synthesizes β–carotene, a pro-vitamin A in the endosperm. So the appearance of grain is yellow and therefore, called "**Golden Rice"** (Ye *et al*.,2000; Beyer *et al.,* 2002; Paine *et al*., 2005). The dietary β–carotene can be converted into vitamin A which is very important for normal development of humans. Vitamin A deficiency causes night blindness or total blindness in children, besides other diseases.

2. **Maize lines** have been developed by genetic engineering. There is 100-fold enhancement in lysine content of the endosperm of these genetically engineered maize lines.

Functions of endosperm

-Endosperm supports early seedling growth.

-It is nutrition of embryo during its early stages of development. (from zygote to globular embryo).

-Apart from being a nutritive tissue, endosperm also regulates the precise mode of embryo development.

In angiosperms, where endosperm is present, it may either be consumed or may persist in mature seeds. So it is the endosperm, on the basis of which seeds can also be categorized into two categories:

1. Non- endospermic seeds (ex-albuminous seeds):

In plants where the entire endosperm consumed or utilized in the nutrition of the developing embryo, the mature seeds thus formed are without endosperm. Such seeds are termed as non-endospermic seeds. Example-Seeds of beans, peas etc. The non-endospermic seeds store their food material in cotyledons.

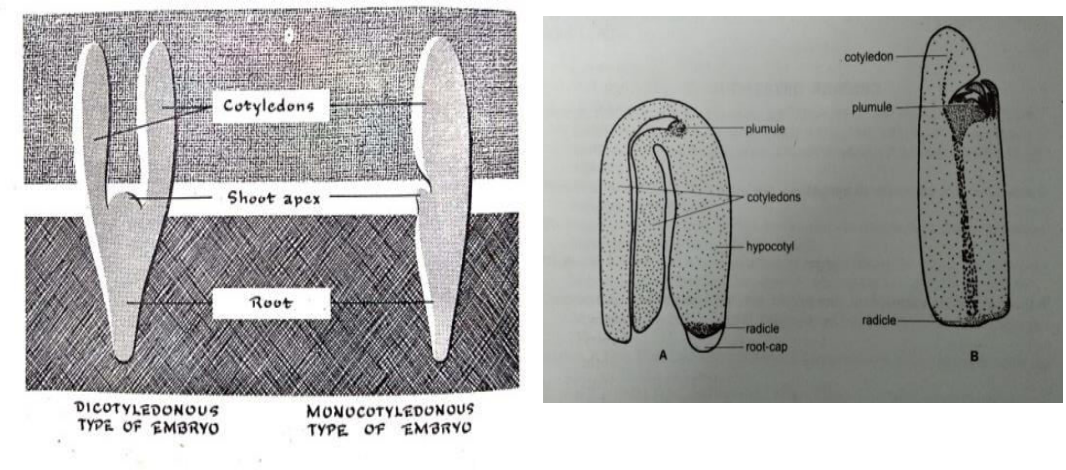
2. Endospermic seeds (albuminous seeds):

In plants where the seeds retain endosperm even at maturity and do not consume the endosperm completely during the development of embryo. Such seeds are said to be endospermic seeds. It can also be said that e**ndosperm** persist in mature seeds and continue to support the growth of embryo during seed germination and even after germination in the establishment of young seedlings. Example- seeds of cereals, coconut, castor etc.

4.6- EMBRYO DEVELOPMENT

After fertilization, a series of changes occur in the ovule and finally seed is formed. Side by side with the development of the endosperm, the zygote develops into the embryo after a period of rest.

The process of development of **mature embryo from zygote** is called **embryogenesis or embryogeny**. Embryo has the potential to develop into a complete plant. Fig. 4.19 A and B shows a typical dicotyledonous embryo and monocotyledonous embryo, respectively.



A **B**

Fig. 4.19*: A typical dicotyledonous embryo and monocotyledonous embryo, First picture (**A**) showing differences between the organography of the embryo in dicotyledons and monocotyledons (source: Comparative Morphology of Vascular Plants by Foster and Gifford) and second picture (**B**) showing median longitudinal section of mature dicotyledonous embryo of Capsella bursa pastoris and monocotyledonous embryo of Hydrilla verticillata (after Maheshwari and Johri, 1950)*

The **dicotyledonous embryo** as the name reflects, has a pair of lateral cotyledons between the bases of which is situated the rudimentary terminal shoot apex or we can also describe it as two cotyledons attached laterally to an embryonical axis. In contrast, the typical embryo in monocotyledons develops a single, terminal cotyledon, and the shoot apex appears lateral in position or we can also describe it as the embryonical axis has a single cotyledon at its apex. Due to this organographic difference, it is very easy to distinguish the two types of embryos. Both dicotyledons and monocotyledons begin embryo development in the same way but there is considerable difference during later differentiation.

Embryogeny

In all angiosperms the embryogenesis starts with the division in zygote and in most of the angiosperms the zygote divides to develop a two celled **proembryo** by forming a transverse wall. A large cell near the micropyle is termed the **basal cell** and the small cell facing towards the centre of the embryo sac is called the **terminal cell or apical cell**.

Rarely the division of the zygote may be vertical as reported in Loranthaceae or oblique as in *Triticum* sp.

There is no fundamental difference in the early stages of development. So early developmental patterns of embryo are common in monocotyledons and dicotyledons and the development is similar till the globular stage. Differences appear when the initials of plumule and cotyledons are laid down.

From the 2-celled stage until the initiation of organs, the embryo is commonly known as proembryo.

Proembryo:

In a two–celled **proembryo,** having large **basal cell** towards micropyle and small centre facing **terminal cell or apical cell**, the basal cell forms the suspensor and may or may not contribute in rest activities so sometimes called as **suspensor cell**, whereas terminal cell is responsible for further development of embryo so called **embryo (or embryonal) cell**.

Divisions in Proembryo:

-The basal cell (cb) either remains undivided or undergoes transverse division to form two cells, m and ci.

-The apical cell divides and depending on whether this division is transverse or vertical and resulting 4-celled proembryo is linear or T-shaped, respectively.

-In a **linear proembryo**, the two daughter cells of apical cell (l and l_1) by two vertical divisions at right angle to each other, give rise to **an octant** with two superposed tiers of four cells each (Fig. 4.20 A).

-Similar **octant** with two superposed tiers is formed by the **T-shaped proembryo** but here by one transverse division and one vertical division (Fig. 4.20 B). T-shaped proembryo may form a different octant having eight cells **in the same tier**; an axial quadrant is surrounded by four peripheral cells (Fig. 4.20 C).

So two types of octant configurations occur in angiosperms (in monocotyledons as well as in dicotyledons)-

1- the component cells are present in two superposed tiers of 4-cells each. Example- *Beta, Poa, Capsella, Sagittaria*

2- all 8- component cells are present in a single tier. Example- *Lactuca, Muscari*

Destiny of cells becomes determined at this octant stage of the proembryo.

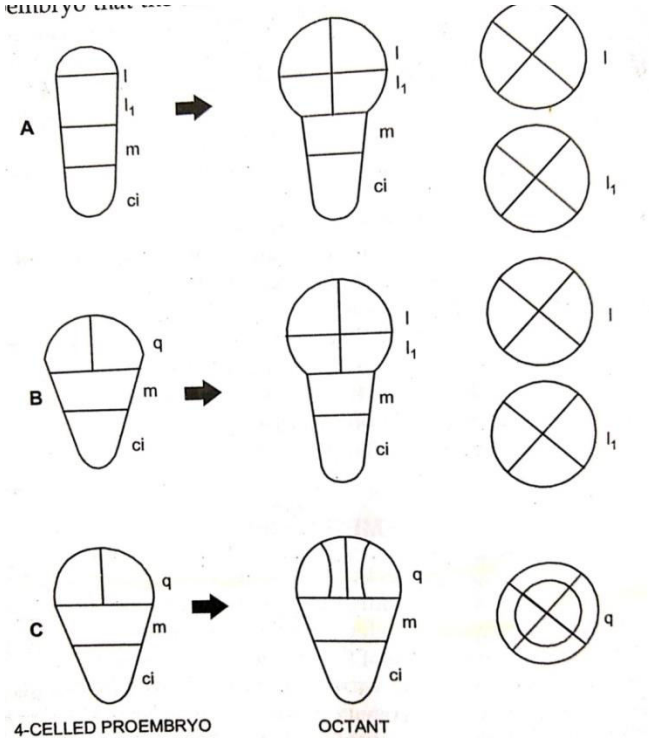


Fig. 4.20 *Formation of two different types of octants. On the extreme right are transverse sections of the tiers l, l1and q of the octants (after Swamy, 1962)*

Types of embryo development:

On the basis of plane of division of the terminal cell (also known as apical or embryo cell) in the 2-celled pro-embryo and the contribution of the basal cell and terminal cell in the formation of embryo proper, **six types of embryogeny** (embryo development) have been reported (Johansen 1950; Maheshwari 1950) in angiosperms (Fig. 4.21).

1. Onagrad or Crucifer type
2. Asterad type
3. Solanad type
4. Caryophyllad type
5. Chenopodiad type
6. Piperad type

A. The **terminal cell** of 2-celled pro-embryo **divides longitudinally**

(1)The basal cell plays only a minor role or none in the subsequent development of the embryo proper----------**Onagrad or Crucifer type** Example- Annonaceae, Brassicaceae, Onagraceae, Pedaliaceae, Ranunculaceae, Scrophulariaceae

(2) The basal cell and terminal cell both contribute to the development of embryo proper---------- **Asterad type** Example- Asteraceae, Balsaminaceae, Violaceae, Vitaceae

B. The **terminal cell** of 2-celled pro-embryo **divides transversely**

I. The basal cell plays only a minor role or none in the subsequent development of the embryo proper

(3) The basal cell usually forms suspensor----------**Solanad type** Example- Campanulaceae, Linaceae, Solanaceae, Theaceae

(4) The basal cell undergoes no further division and the suspensor, if present, is always derived from the terminal cell----------**Caryophyllad type** Example- Caryophyllaceae, Crassulaceae, Haloragaceae

II. (5) The basal cell and terminal cell both contribute to the development of embryo proper----------**Chenopodiad type** Example- Boraginaceae, Chenopodiaceae

These five types of embryogeny are reported in those plants where first division of the zygote (oospore) is transverse forming terminal and basal cell.

Johansen (1950) has also reported a **sixth type of embryogeny**, called **Piperad type**. In this type the first division of the zygote is vertical. Example- Loranthaceae, Piperaceae.

Although the embryogenesis pattern is same throughout a family but there are few reports where same species shows more than one well established pattern of embryo development. Example- *Anemone rivularis* exhibit *Solanad Type* as well as *Crucifer Type* of embryogeny.

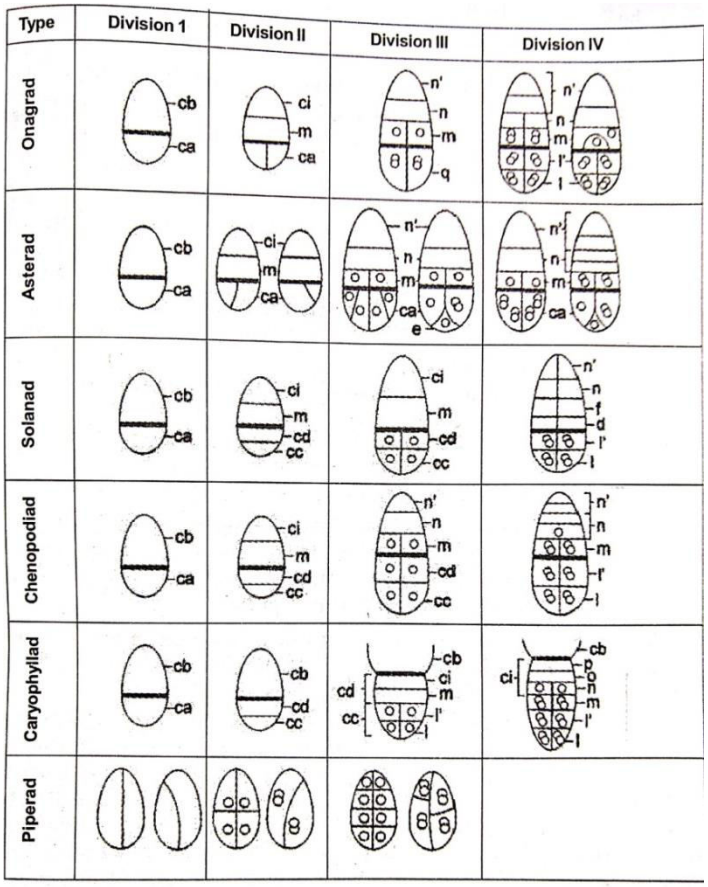


Fig. 4.21- *Main types of embryogeny according to the classification of Johansen (1950)*

- The process of development of **mature embryo from diploid oospore** is called **embryogenesis.**
- In majority of angiosperm plants, the first division of the zygote is transverse. Rarely is it vertical.
- Six types of embryogeny was reported among angiosperms by Johansen (1950)- *Onagrad Type or Crucifer Type, Asterad Type, Solanad Type, Caryophyllad Type, Chenopodiad Type and Piperad Type*

Development of dicotyledonous embryo

It can be studied by going through following examples:

(I) The classical example is ***Capsella bursa-pastoris*** (Shepherd's purse) of Cruciferae (Fig. 4.22). The ovule is campylotropous so the embryo sac and the later developed endosperm as well as embryo are horseshoe-shaped. Here the development of embryo is **Onagrad or Crucifer type.**

The first division (transverse) of the zygote yields a **terminal and a basal cell** (t and b, Fig. 22B). As a result of this a **two-celled pro-embryo** is formed. Then the basal cell divides transversely and a vertical division follows in the terminal cell (Fig. 22C-E). As a result, a four-celled **pro-embryo** develops.

> The larger **basal cell** at the micropylar end is called suspensor cell because it contributes to the formation of **suspensor**. The smaller one, away from it termed as **terminal cell** or **embryo cell** because it contributes to the formation of **embryo**.

Terminal cell undergoes a vertical division forming two cell and each of the two terminal cells again divides vertically at right angle to the first one, resulting in a quadrant of cells (Fig. 4.22 F-J). Transverse division then in each of these four cells yields the octant stage of the embryo (Fig. 4.22K).

All the eight cells now divide periclinally into an outer layer of eight cells and an inner eight cells. So in the young embryo proper eight external cells undergo anticlinal divisions to produce the embryonic surface layer or **dermatogen** while eight internal cells, will develop the ground meristem and procambial system of the hypocotyls and cotyledons after gradual differentiation (Fig. 4.22 L-N). Inner eight cells divide and redivide to form a mass of cells, of which cells of the central portion divide at a faster rate as compared to peripheral cells thus demarcating a central mass of smaller cells (**pleurome**) from peripheral layer of larger cells (**periblem**). Pleurome develops conducting tissue while periblem develops ground tissue in future.

During the formation of octant stage of the embryo proper, the suspensor develops from the two upper cells of the proembryo by transverse divisions (Fig. 4.22 F-M). The suspensor cell next to the micropylar end of the embryo sac usually fails to divide, but instead, progressively enlarges to form a very conspicuous vesicular cell. A variable number of transverse divisions in another cell (2 celled suspensor) and its descendents produce 5-7 additional suspensor cells. The lowermost cell of the suspensor was originally termed as hypophysis by Hanstein. This cell, by transverse and longitudinal divisions, produces two four-celled tiers of cells (Fig. 4.22 N-Q). Derivatives of the lower tier contribute to the cortex of the embryonic root, and derivatives of the tier near the suspensor form the earlier cells of the root cap and the adjacent root epidermis.

The young embryo of *Capsella*, as a result of well-coordinated sequence of cell divisions, now consists of a spherical group of cells attached to a filamentous suspensor (Fig. 4.22 Q).

The paired cotyledons arise as two ridges of tissue derived from the distal tier of the embryo and a few cells situated between the bases of the cotyledon primordial remain undifferentiated and constitute the future shoot apex (plumule) of the embryo (Fig. 4.22 and 4.23). During cotyledon initiation, the division and differentiation of cells in the lower tier of the embryo gradually produce the young axis or hypocotyl of the embryo. At this stage the embryo is somewhat heart-shaped. Continued enlargement of the hypocotyls and cotyledons results in a pronounced curvature of the cotyledons which lie parallel to the axis of the embryo in the mature seed (Fig. 4.22 and 4.23).

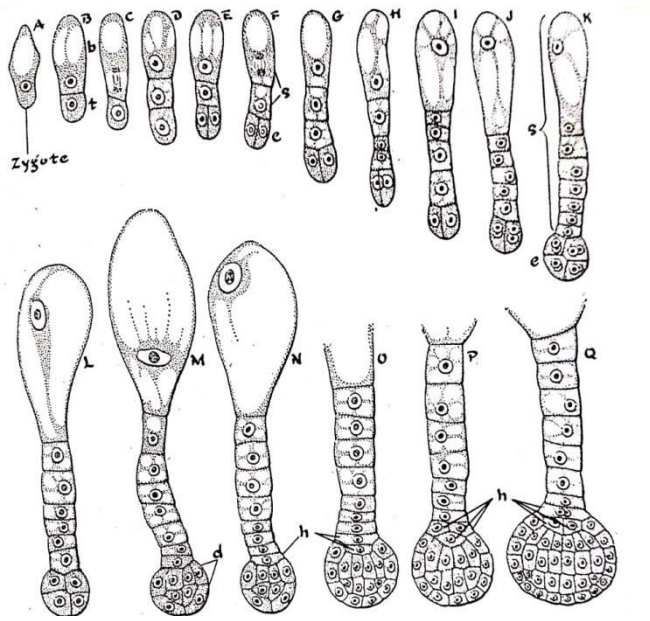


Fig. 4.22 *Early embryogeny in Capsella bursa-pastoris.* ***A-B*** *division of zygote into terminal (t) and basal cells (b);* ***C-D,*** *development of three-celled proembryo;* ***F-H*** *formation of suspensor cells (s) and division of terminal cells resulting in the quadrant (**J**) and octant (**K-L**) stage of embryo proper (e);* ***M*** *origin of dermatogens (d) representing the surface cells of epidermis of the embryo;* ***N-Q*** *origin and division of hypophysis cell (h) and continued increase in number of surface and internal cells in the globular embryo [Redrawn from Soueges, Ann Sci Nat Bot X.1:1, 1919]*

-The suspensor helps in pushing the embryo in the endosperm.

-The first cell of the suspensor (towards micropyle) called **haustorium or vesicular cell**.

Upon germination the plumule forms the shoot and the radical gives rise the root system. The reserve food material in the cotyledons is used in the establishment of young seedlings.

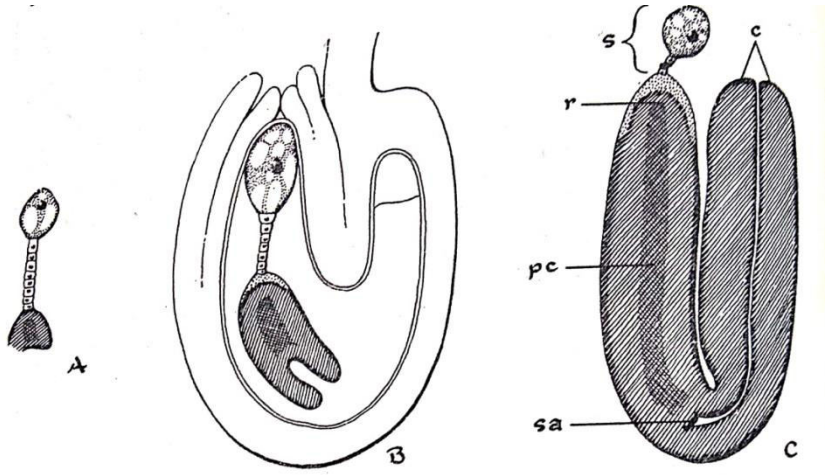


Fig. 4.23 *Late stage in embryogeny in Capsella.* ***A*** *the cordate form of a longisection of an embryo at stage of initiation of cotyledons;* ***B*** *longisection of developing seed showing orientation and general structure of an embryo with two cotyledons;* ***C*** *longisection of an embryo from a mature seed (c, cotyledons, pc, procambium, r, tip of root, s, suspensor, sa, shoot apex [A and C redrawn from Schaffner, Ohio Nat. 7:1, 1906; B after Bergen and Caldwell and redrawn from A Textbook of General Botany, Ed.4, by R.M. Holman and W.W. Robbins.N.Y.: John Wiley and Sons. Inc, 1951]*

Dicot embryo
-A typical dicotyledonous embryo consists of an embryonal axis and two cotyledons.
-Above cotyledons level, embryonal axis is called epicotyl which forms shoot.
-Below cotyledons level, embryonal axis is called hypocotyl which forms radicle.

(II) ***Ceratocephalus falcatus*** **(Ranunculaceae)-Bhandari and Asnani 1968. Here embryogeny is** *Onagrad Type* (Fig. 4.24).

1. Zygote divides **transversely** to form a **two-celled pro-embryo** having a large basal cell at the micropylar end and a small apical cell away from it.
2. Basal cell divides transversely, forming two superposed cells *i* and *m*.
3. The apical cell undergoes a vertical division giving rise to two juxtaposed cells (daughter cells). So a T-shaped, 4-celled proembryo (**quadrant)** is formed.
4. One of the two superposed cells i.e. *i* divides transversely giving rise to *n* and *n'*. These two cells divide further forming a linear row of 3- or 4- celled suspensor, a ephemeral structure mainly concerned with the nutrition of the proembryo.
5. Other superposed cell *m* and its derivatives divide by a vertical division to form 4-6 cells. Oblique periclinal division in each of these cells result in an inner set of cells (the initials of root apex) and an outer set of cells (the initials of root cap).

6. Daughter cells of the apical cell divide by another vertical division at right angle to the first division resulting into quadrant. Cells of this quadrant divide transversely to form octant. All the resulting eight cells arranged in two tiers (l, l_1) of four cells each.
7. Cells in these two tiers (l, l_1) further divide vertically to form a **globular proembryo**.
8. **Fate of product cells of l**- Periclinal division in the peripheral cells of the globular proembryo demarcate a single layered dermatogen, the future epidermis and inner cells differentiate the initials of plumule and the two cotyledons. Cotyledons flank the plumule on either side.
9. Growth in the cotyledonary zones is much faster than in the plumular zone. Due to this activity, the plumule is enclosed at the base of the two cotyledons in the mature embryo.
10. **Fate of product cells of l_1**- These cells finally forms the hypocotyl-radicle axis.

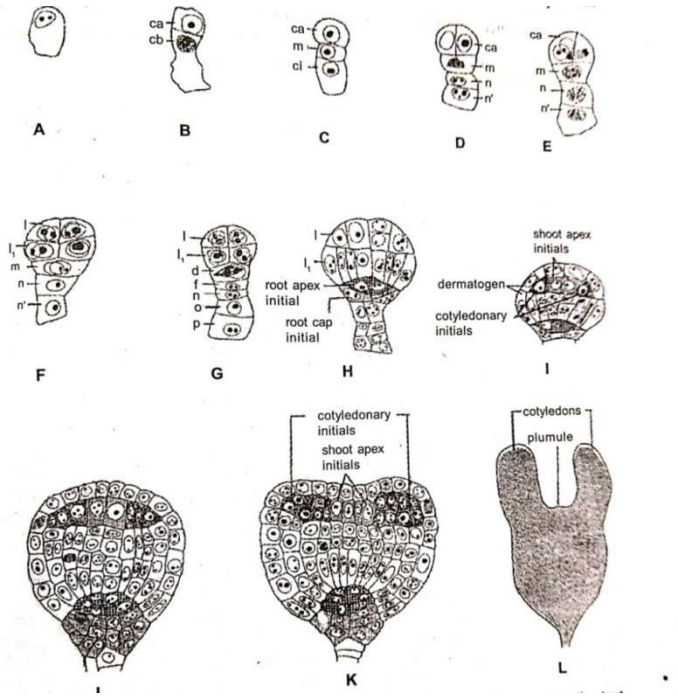


Fig. 4.24- *Development of embryo in Ceratocephalus falcatus (after Bhandari and Asnani, 1968)*

Development of monocotyledonous embryo

Development of embryo up to the octant stage is almost similar in monocotyledons and dicotyledons. There is no essential difference between the embryogeny of both but as a single cotyledon develops in monocotyledons, there is some difference at later stages.

Wardlaw (1955) remarked: "In the dicotyledonous embryo, the plumule is typically distal and is situated symmetrically between two equivalent cotyledons: in the

monocotyledonous embryo, the shoot apex occupies a lateral position in the somewhat cylindrical embryo and cotyledon is terminal."

According to Lakshmanan (1972) the main difference between embryos of two lies in the number of cells of the terminal quadrant of a proembryo which contribute to the formation of cotyledon(s) and epicotyl:

So we can say that **the number of cells of the terminal quadrant of a proembryo** participating in the formation of cotyledons and epicotyl decides whether it will be **monocotyledonous or dicotyledonous embryo.**

Where the number of cells forming cotyledon(s) is same, the relative position of cells in the quadrant may be different.

In dicotyledonous embryo- two opposite cells of the terminal quadrant give rise to the two cotyledons.

In monocotyledonous embryo- Number of cells involved in the formation of cotyledons is variable.

i-All the four cells except a few cells derived from one of the quadrant cells---------- in the Philydraceae (Fig. 4.25 B)

ii- Three cells of the quadrant----------in the Iridaceae, Pontederiaceae, Sparganiaceae (Fig. 4.25 C)

iii- two adjacent cells----------in the Amaryllidaceae, Hydrocharitaceae, Potamogetonaceae(Fig.4.25D)

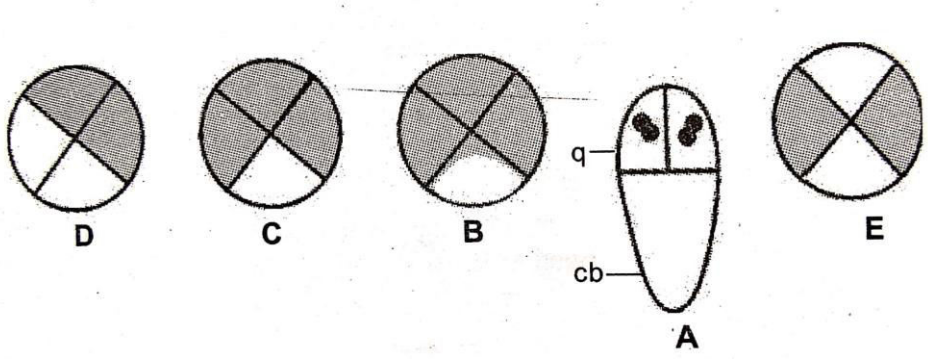


Fig. 4.25- *Derivation of cotyledons in monocotyledons (B-D) and dicotyledons (E).*

A.Proembryo at the quadrant stage. B-E. T.S. of the terminal tier. Stippled parts of the quadrant represent the portion contributing to the formation of cotyledon(s). In monocotyledons, more than three sectors (B), three sectors (C) or two adjacent sectors (D) form the sinbgle cotyledon, whereas in dicotyledons, two opposite sectors (E) develop into a pair of cotyledons [after Lakshmanan, 1972]

Development study on *Najas lacerata* (a monocot) according to Swamy and Lakshmanan (1962)**.** Here the development of embryo is ***Caryophyllad type.***

1. The zygote divides into two cells by transverse division- a large basal cell (*cb*) and small apical (*ca*) cell (Fig. 4.26 A).
2. There is no further division in the basal cell and it enlarges to form a single-celled haustorium. Only the apical cell participates in the development of embryo.
3. The apical cell divides transversely into two cells- *c* and *d.*
4. The cell *d* divides into two cells. This division is again transverse.

As a result of this a linear proembryo of four-cell *c, m, ci, cb* is formed (Fig. 4.26 B).

5. Vertical division takes place in the two distal cells *c* and *m* at right angles to each other resulting in two superposed tiers (*q, m*) of four cells each (Fig. 4.26 C).
6. Transverse division occurs in *ci* forming *n* and *n'* cells (Fig. 4.26 D).
7. *n divides* vertically while *n'* forms two cells *o* and *p* by transverse division (Fig. 4.26 E).
8. *p* undergoes another transverse division producing *h* and *s* cells.
9. Quadrant *q* divides by periclinal division cutting a four-celled dermatogen surrounding the four axial cells.
10. Cells of *m* tier divide by vertical and transverse divisions and become two tiered. At this stage proembryo is **slightly spherical** (Fig. 4.26 F). Now onward it elongates due to transverse divisions in the tiers *m* and *n*.

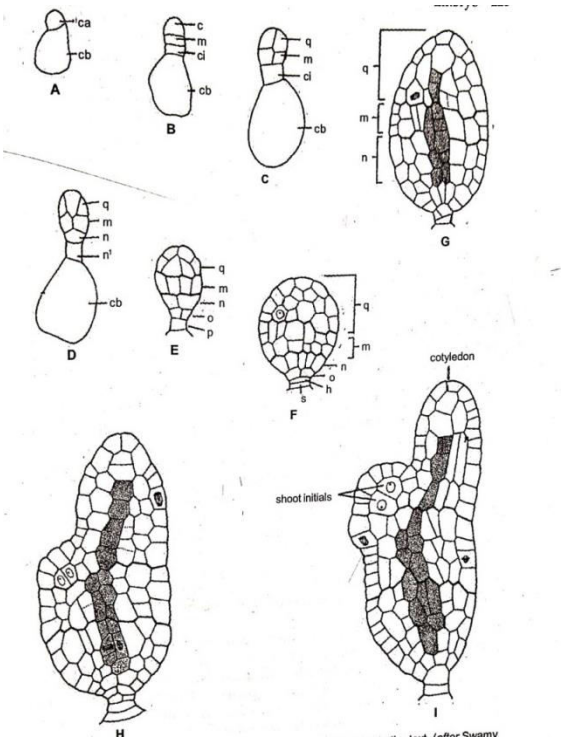


Fig. 4.26- *Development of embryo in Najas lacerata*

11. When embryo becomes oval, the **central core of cells** in the tiers ***q, m* and *n*** differentiates into **plerome initials** (Fig. 4.26 G).
12.At the eight-celled stage in tier *q* (4 axial cells and 4 circum axial cells), three of the axial cells divide faster than the fourth one and disturb the symmetry of the proembryo due to which its top becomes notched (Fig. 4.26 H).
13. The rapidly growing portion of the tier *q* forms the single cotyledon and the slow growing tissue derived from the fourth axial cell gives rise to the initials of epicotyls (Fig. 4.26 I).
14. The radical is organized from the derivatives of *n.*
So, in *Najas*, the single cotyledon and the epicotyls are distinctly terminal structures.

(II) Another example is of ***Luzula forsteri* of Juncaceae** for describing the development of monocotyledonous embryo. Here the development of embryo is **Onagrad or Crucifer type**.
As usual the early development of dicot and monocot embryos is similar upto **octant stage**. Later on differentiation starts.
1. The zygote elongates followed by transverse division to form basal and terminal cells.
2. The basal cell (towards micropylar end) produces a large swollen, vesicular suspensor cell. It may function as haustorium.
3. The terminal cell divides by another transverse wall to form two cells.
4. The top cell after a series of divisions forms plumule and a single cotyledon.
5. Cotyledon called scutellum, grows rapidly and pushes the terminal plumule to one side. The plumule lies in a depression.
6. The middle cell after many divisions forms hypocotyl and radicle. It also adds a few cells to the suspensor.
In some cereals both plumule and radicle get covered by sheaths developed from scutellum called coleoptile and coleorhiza respectively.

(III) One more example is ***Grass embryo:*** The monocotyledonous embryo of grasses is very different from that of other monocotyledons in its mature structure as well as development.

Development of embryo in *Triticum* according to Batygina (1969)

According to Batygina all the four species of *Triticum* show a fixed pattern of embryogeny- ***Graminad type***

1. The zygote divides into two cells - a large basal cell (*cb*) and small apical cell (*ca*) but here this division is by lying down of **an oblique wall** instead of transverse wall (Fig. 4.27 A-C).

2. The basal cell again divides obliquely forming cells *ci* and *m.* The upper end of the wall formed during this division connects with the wall separating *ca* and *cb* (Fig. 4.27 D).

3. The third division occurs in the cell *ca,* in a plane perpendicular to the first division of the zygote (Fig. 4.27 D).

Thus a T-shaped proembryo (4-celled) is formed. Here the orientation of the walls is very different from the T-shaped tetrad in either dicots or in other monocots. This characterizes **the wheat embryogeny**.

4. The cell *ci* divides by a wall at right angles to the wall between *ci* and *m,* resulting in the formation of cells *n* and *n'* (Fig. 4.27 E).

5. Divisions of the daughter cells of *ca* are in the same plane as the first division in *ca* but at right angle to it, forming the typical quadrant *q* (Fig. 4.27 F).

6. Vertical divisions take place in the cell. Further divisions occur in various planes. 7. Organogenesis sets in at 16-32 celled stage of the proembryo. The first organ to be initiated is the single cotyledon or scutellum (Fig. 4.27 G-I).

8. With further development a constriction appears opposite to the scutellum demarcating it from the rest of the embryo (Fig. 4.27 J).

9. Then primordial of coleoptiles appears followed by shoot apex close to the notch (Fig. 4.27 K).

10. The radical differentiates endogenously in the central zone of the embryo (Fig. 4.27 L). Fig. 4.27 M is showing mature embryo.

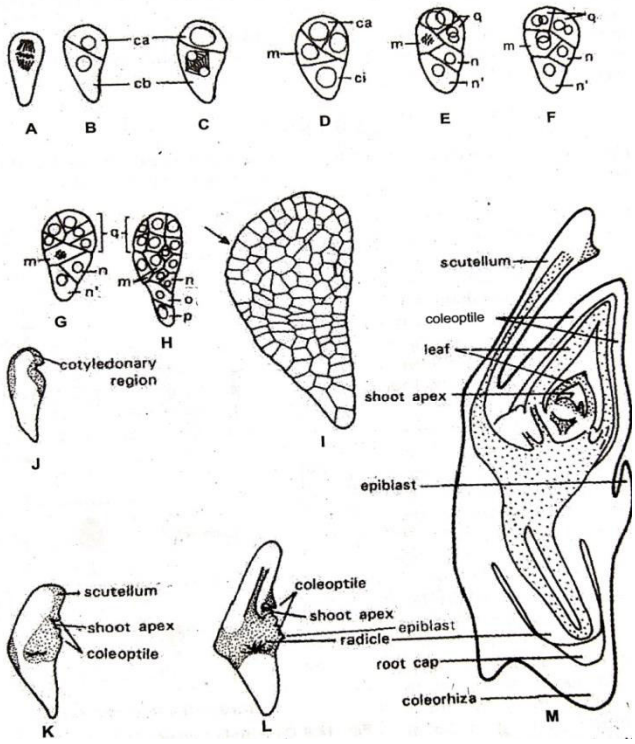


Fig. 4.27- *Development of embryo in Triticum*

(IV) Development of embryo in *Sagittaria sagitifolia* - Different stages of development of embryo in *Sagittaria* are clearly visible in Fig. 4.28 and by going through this figure and its legends you can easily understand each and every event of embryo development.

Structure of Monocot Embryo:

The embryo of monocotyledons (Fig. 4.19) has only **one cotyledon.**

A mature embryo of *Triticum* member of grass family (Gramineae or Poaceae), has single cotyledon, called **scutellum** (Fig. 27M). It is situated towards lateral side of embryonal axis. This axis below the level of scutellum is the **radicle** and root cap enclosed in a sheath called coleorhiza. The part of axis above the level of scutellum is called **epicotyl.** It has shoot apex and few leaf primordia enclosed in a hollow foliar structure called coleoptile. On one side the coleorhiza there is a small outgrowth called epiblast which represents rudiments of second cotyledon.

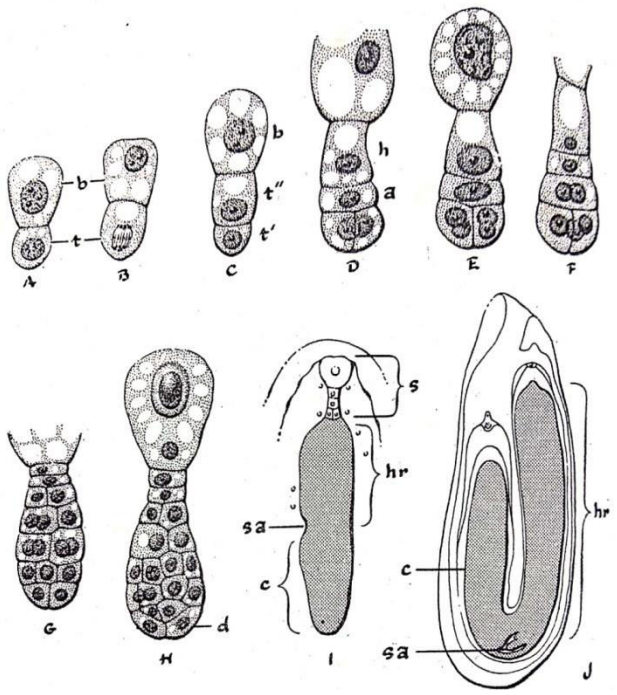


Fig. 4.28 *Early embryogeny in* ***Sagittaria sagitifolia****. A. The zygote of* ***Sagittaria*** *divides by transverse wall into a terminal (t) and basal cell (b). (B-C). Basal cell divides no further, but progressively enlarges during embryogeny to form a conspicuous vesicular cell. Meanwhile the terminal cell divides transversely into t' and t'' and the proembryo at this stage consists of three superposed cells. (D). Transverse division of t'' forming cells a and h, and vertical division of t'. (E). Cells derived from t' again divide longitudinally (quadrant stage) (F). Vertical division of cell a and transverse division of cell h (G). The four terminal cells divides transversely- Octant stage (H). Origin of dermatogens (d) (I-J). Late stage of embryogeny. (I).Longisection of embryo-c:terminal cotyledon; sa: shott apex; hr: hypocotyls-root; s: suspensor; (J) longisection of curved embryo in mature seed [A-H redrawn from Soueges, Ann. Sci.Nat. Bot.X.13:353, 1931; I-J, redrawn from Schaffner, Bot. Gaz.23:252,1897]*

Suspensor

The suspensor is an ephemeral structure, found at the radicular end of the proembryo and grows much faster than the embryo during early stages of embryo development. It attains its maximum size at the globular or early heart shaped stage.

Function: The suspensor anchors the embryo to the embryo sac and pushes it deep into the endosperm so that the embryo lies in a nutritionally rich environment. The suspensor is also actively involved in the absorption of nutrients from various ovular and extra-ovular tissues and transporting them to the embryo proper.

Suspensor is exceptionally long in the Loranthaceae. Why?

Here the embryo sac grows up to various heights in the style and the egg is fertilized at the tip of the embryo sac in the style. The endosperm is formed inside the ovary. That's why the suspensor is exceptionally long in the Loranthaceae so that it is able to bring the embryo down into the endosperm for nutrition.

Monocot embryo

1. A typical monocotyledonous embryo consists of an embryonal axis and one cotyledon.
2. In grasses, this cotyledon is called **scutellum.**
4. Embryonal axis at its upper end is called epicotyls which forms shoot.
5. Embryonal axis at its lower end is called hypocotyl which forms radicle.
6. The root tip is covered with a sheath (coleorhiza).

4.7 SUMMARY

In this unit we have discussed about fertilization, pathway of pollens to their destination for fertilization. By going through this unit it is now clear that the phenomenon of double fertilization is a characteristic feature of angiosperms. After that we have also learnt post fertilization developmental processes including endosperm as well as embryo development. Along with these light have been thrown on embryo development in both monocots and dicots by taking different examples. Therefore, summary of all the topics covered in this unit is given in the following key points:

- Once the pollen grain reaches the receptive stigma, as a result of pollination, it germinates producing a long slender pollen tube. Two male gametes and the tube nucleus migrate into the pollen tube which now represents the mature male gametophyte.
- Pollen tube penetrates the stigmatic tissue and pushes its way through the style and then down to the wall of the ovary.
- After reaching the ovary, the pollen tube may enter into the ovule through micropyle (porogamy), chalaza (chalazogamy) or integuments (mesogamy).
- Irrespective of the route of the entry of the pollen tube into the ovule, it always enters the embryo sac from the micropylar end.
- After entering into the embryo sac, the tip of the pollen tube bursts and the two male gametes are released.
- Out of the two male gametes, one male gamete fuses with the egg nucleus forming zygote (2n). Second male gamete fuses with two polar nuclei, resulting into primary endosperm nucleus (3n). This phenomenon is termed as double fertilization.

- After series of divisions primary endosperm nucleus forms endosperm. Endosperm is very nutritive tissue that nourishes the developing embryo.
- Depending upon mode of development, three types of endosperm has been recognized i.e., nuclear, cellular and helobial.
- If mature endosperm shows any degree of irregularity and unevenness in its surface, then it is called ruminate endosperm.
- Zygote forms embryo.
- The process of development of **mature embryo from zygote** is called **embryogenesis.**
- In majority of angiosperm plants, the first division of the zygote is transverse. Rarely it is vertical.
- On the basis of plane of division of the terminal cell in the 2-celled pro-embryo and the contribution of the basal cell and terminal cells in the formation of embryo proper, six types of embryogeny have been reported by Johansen (1950) among the angiosperms. These are Onagrad or Crucifer, Asterad, Solanad, Caryophyllad, Chenopodiad and Piperad types.
- A typical dicotyledonous embryo consists of an embryonal axis with two cotyledons.
- A typical monocotyledonous embryo consists of an embryonal axis and one cotyledon.

4.8 GLOSSARY

Albuminous seeds: mature seeds with endosperm

Chalazogamy: entry of pollen tube through the chalazal end

Coleoptile: a plumule sheath found in monocot seeds

Coleorrhiza: a sheath surrounding radicle found in monocot seeds

Cotyledon: an embryonic leaf of the embryo

Dicot embryo: embryonal axis having two cotyledons

Embryo: a young sporophytic plant inside the seed

Embryogenesis: The process of development of mature embryo from diploid oospore

Endosperm: a nourishing tissue in seed bearing plants

Epiblast: a small outgrowth found opposite to scutellum in the embryo of some members of Gramineae

Epicotyl: the stem of an embryo (or seedling) above the cotyledons

Ex-albuminous seeds: mature seeds without endosperm

Fertilization: process of fusion of two dissimilar reproductive units, called gametes

Hypophysis: one of the cells, lowest one in suspensor

Integument: an envelope surrounding the nucellus (in ovule)

Liquid syncytium: clear fluid (here watery liquid endosperm) in which numerous nuclei float

Megasporogenesis: development of megaspore from megaspore mother cell

Mesogamy: entry of pollen tube through the integument or funiculus
Monocot embryo: embryonal axis having one cotyledon
Monocotyledon: a flowering plant with one cotyledon in its seed
Porogamy: entry of pollen tube through the micropyle
Ruminate endosperm: If mature endosperm shows any degree of irregularity and unevenness in its surface, then it is called ruminate endosperms.
Scutellum: specialized monocot's cotyledon
Suspensor: the part of the embryo which connect the main part of the embryo to the embryo sac
Triple fusion: A unique feature of flowering plants wherein fusion of one male gamete with the two polar nuclei to form primary endosperm nucleus
Zygote: the fusion product of an egg and a male gamete, i.e., a fertilized egg

4.9 SELF ASSESSMENT QUESTIONS

4.9.1 Multiple choice questions

1. Entry of pollen tube into the ovule through micropyle is called
a. Chalazogamy b. Porogamy
c. Mesogamy d. None

2. Entry of pollen tube into the ovule through the chalazal end is called
a. Chalazogamy b.Porogamy
c. Mesogamy d. None

3. Entry of pollen tube into the ovule through the funiculus or integuments is called
a. Chalazogamy b. Porogamy
c. Mesogamy d. None

4. Product of syngamy is
a. Zygote b. Oosphere
c. Primary endosperm nucleus d. Embryo

5. Product of triple fusion is
a. Zygote b. Oosphere
c. Primary endosperm nucleus d. Embryo

6. Zygote develops into
a. Endosoerm b. Polar nuclei
c. Embryo d. Egg

7. Endosperm is formed from

a. Primary endosperm nucleus b. Secondary nucleus

c. Egg d. Embryo

8. Two- celled proembryo has

a. Apical cell and terminal cell b. Suspensor cells only

c. Spore cells d. Basal cell and terminal cell

9. In dicot embryo development, the hypophysis is formed from

a. Terminal cell b. Embryo cell

c. Suspensor cell d. None

10. The development of several embryos within the same ovule is known as

a. Embryony b. Polyembryony

c. Both d. None

11. Double fertilization in flowering plants produces

a. Diploid oosphere and diploid endosperm b. Diploid zygote and triploid primary endosperm nucleus

c. Diploid zygote and triploid oosphere d. Diploid endosperm and triploid zygote

12. Endosperm

a. Provide nutrition to the embryo b. First cell of male gametophyte

c. Produced by syngamy d. Product of meiosis in microspore mother cell

4.9.2 Short answer questions

1. Q: On the surface of stigma the pollen hydrates. What does this reflect?
2. Q: What is the role of enzyme cutinase in pollen tube?
3. Q: Who discovered chalazogamy?
4. Q: Is the way of entry of pollen tube to ovule and embryo sac same?
5. Q.What is the difference between syngamy and triple fusion?
6. Q. Why zygote is diploid?
7. Q. Define endosperm.
8. Q.What is the main role of endosperm?
9. Q. Name the families where endosperm is absent.
10. Q.What is double fertilization?
11. Q. Who discovered the process of fertilization in flowering plants?
12. Q.Who discovered the phenomenon of double fertilization?
13. Q. Are angiospermic and gymnospermic endosperms same?

14. Q. Name types of endosperms.
15. Q. Which type of endosperm is common in angiosperms?
16. Q. What is liquid syncytium?
17. Q. What is embryogenesis?
18. Q. Name the different types of embryo developments.
19. Q. Differentiate dicot and monocot embryo.
20. Q. Why basal cell is known as suspensor cell and terminal cell is known as embryo cell?

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4.12 TERMINAL QUESTIONS

Long answer type questions

1. Q. Define briefly the endosperm development in *Cocus nucifera.*
2. Q. Describe in detail the post fertilization developments in Angiosperms.
3. Q. Explain the development of different types of endosperms in angiosperms.
4. Q. Discuss different types of embryo development
5. Q. Describe the embryo development in dicots with the help of labeled diagrams.
6. Q. Describe the embryo development in monocots with variations reported in different plants.

UNIT- 5: SEED AND FRUIT DEVELOPMENT AND FRUIT TYPES

5.1 OBJECTIVES

- To study the structure of seed.
- To study the development of the seed.
- To study the factors affecting seed development
- To study the types of seeds.
- To study the development of the fruit.
- To study the different types of fruits.

5.2 INTRODUCTION

The seeds and fruits are the results of fertilization or sexual reproduction in plants. The ovary in angiosperms develops into the fruit whereas the ovules become the seeds enclosed within the fruit. Seeds are found both in gymnosperms and angiosperms.

Once pollination and fertilization occur, the mature ovule begins to differentiate into a seed which consists of seed coat, cotyledons and endosperm whereas the ovary begins to differentiate into the fruit. The outer wall of the ovary begins to differentiate into the pericarp whereas the seed develops within the fruit itself.

Seeds can be classified as endospermic and non-endospermic seeds. Those seeds that utilize the endosperm during the embryo development completely are called as non-endospermic seeds. Those seeds which do not completely utilize the endosperm during the growth of the embryo are called as endospermic seeds

Fruits can be classified in many ways like True and False Fruits; Simple, Aggregate, and Multiple Fruits. Simple fruits are further classified as fleshy and dry fruits depending on their appearance.

5.3 SEED

The seed is a meristematic axis, often associated with a storage tissue and enclosed by an enveloping series of membranes and sometimes stony shells which collectively make up the seed coat. Seed is defined as a mature, integumented megasporangium. All flowering plants bear seeds. In fact seed is a miniature plant. Each seed encloses an inactive embryo. Under suitable conditions the embryo becomes active and germinates to give rise to an adult plant. A typical seed has the following parts.

1. **Seed Coat:** It is protective covering of the seed and is made up of two layers: (a) outer-called Testa which is usually hard and (b) inner-called Tegmen which is thin and papery. There is a small opening at one end of the seed coat, called micropyle through which water enters the seed. The stalk of the seed with which the seed is attached to fruit wall is called funiculus. A large scar is located near the middle of one edge where the seed breaks from the stalk of funiculus, this is called hilum. There is a ridge beyond the hilum opposite the micropyle. It represents the base of the funicuus which is fused with the integuments and is called raphe.

2. Embryo: Embryo is a young plant enclosed in a seed coat. It has two Parts:
(A) Cotyledons: These are leaves of embryo. Their number is either one or two. Sometimes they store food materials and become fleshy. When they do not store food they remain thin and papery. The coty ledons are hinged to an axis (tigellum) at a point called cotyledonary node and open out like a book.

(B) Tigellum: It is the main axis of the embryo. One end of the tigellum is pointed and protrudes out of cotyledons. This lies next to micropyle and is called radicle (rudimentary root). The other end of tigellum is the feathery plumule (first apical bud of shoot). The portion of the axis above the point of attachment of cotyledons is called epicotyl and that below the cotyledonary node is called hypocotyl.

3. Endosperm: It is a food-laden tissue, either present on one side of the embryo or surrounding the embryo on all sides. In some seeds the endosperm is present until maturity. Such seeds are called endospermic (albuminous) seeds. In some seeds it is consumed in young stages by the developing cotyledons and such seeds do not have endosperm at maturity. Such seeds are called non-endospermic (ex-albuminous) seeds.

All parts enclosed by seed coat are called kernel.

5.4 SEED DEVELOPMENT

The seed development process can be divided into four phases.

- Phases I and II comprise cell division and expansion.
- Phase III comprises accumulation of reserves resulting into the increase of seed dry mass.
- Phase IV. Seed moisture loss is intensified.

After fertilization, there is a period of seed structure formation as a result of cell division, expansion and differentiation in which seed structure primordia are formed and future embryo parts can be visualized. During this phase, there is a significant increase in seed size forming the embryonic cells that receive assimilates from the parent plant. During this period, seed moisture content remains constant and high. The significant decrease in seed

moisture content occurs at the end of maturation when changes in cell membrane structure occurs as well as increases in enzyme synthesis in preparation for the successful germination. Recalcitrant seeds usually do not show this transition period between maturation and germination.

Changes occurring during seed development

The following changes occur during seed development:

Seed moisture content: Ovule moisture content at the time of fertilization is approximately 80% (fresh weight basis), both for monocots and dicots. The initial phase of dehydration is slow, and is accelerated from the time the seeds reach maximum dry weight and at that time, seeds possess 35% to 55% moisture content for orthodox monocot and dicot seeds respectively, produced in dry fruits. This decrease in moisture content proceeds until hygroscopic equilibrium is attained. Seeds produced in fleshy fruits have a lower decrease in moisture content than seeds produced in dry fruits. Developing recalcitrant seeds do not show marked changes in desiccation at the end of maturation, possessing moisture contents usually over 60% (fresh weight basis).

Seed size: The fertilized ovule is a small structure with respect to final seed size. Plant species with large seeds can facilitate growth due to their greater protein and lipid reserves, or their more advanced development. Large seeds cannot be physically borne on small plants because of the weight of the seed, which may partly explain the association between plant size and seed size.

Seed dry weight: The developing seeds begin to increase in weight as a result of nutrient accumulation and water uptake. Seed fill is initially slow because cell division and elongation are occurring during this stage. Soon after that the dry mass accumulation increases until seeds reach their maximum dry weight.

Germinability: Seeds of various cultivated species are able to germinate a few days after seed maturation. The percentage of germinable seeds increases during maturation, reaching a maximum around the time when seeds attain maximum dry weight. This is only found in species where dormancy does not occur, because the imbalance in the germination promoters/inhibitors induced during the reserve accumulation period may directly affect seed germinability.

Vigor: Seed vigor changes are usually parallel to nutrient reserve transfer from the parent plant *i.e*. the proportion of vigorous seeds increases during maturation, reaching a maximum near to the seed's maximum dry weight.

Determination of physiological maturity of seed

The identification of the time of physiological maturity has been a controversial subject among different authors studying seed maturation. Among the differing physiological maturity concepts, following three are dominant:

a. Physiological maturity is identified as maximum seed dry matter accumulation
b. Physiological maturity is reached when there is no further significant increase in seed dry weight
c. Physiological maturity occurs when seeds reach maximum dry weight, germination and vigor

Factors affecting seed development

Environmental factors that influence seed development include soil fertility, water, temperature, light, and seed position on the plant.

Soil fertility: In general, plants that have the three major elements (N, P, and K) produce larger seeds than those which do not contain. The increase in seed size is due to an enhanced seed development rate during the seed filling period as a consequence of increased nutrient availability.

Water: Water deficits reduce plant metabolism and seed development. Prolonged droughts and reduced soil water availability cause decrease in seed size, particularly when these effects occur during seed filling. If water deficits occur during flowering, its primary effect is on a reduction in seed number.

Temperature: High temperatures during seed development produce smaller seeds, while low temperatures retard seed growth. Seed germination and vigor are also adversely affected by exposure to low temperatures during development. High temperatures are considered the principal reason for the "forced maturation" of some plants.

Light: The seasonal distribution of solar radiation is a fundamental factor in assuring adequate plant development. In general, reduced light to the parent plant results in smaller seeds.

Seed position on the plant

The position in the inflorescence can affect seed development rate. For example, distal seeds in a wheat spike have slower growth rates and shorter seed filling periods than proximal seeds. Corn seeds at the tip of the ear are smaller than those at the base which has been attributed to inadequate photosynthetic supply.

Seed viability or Longevity of Seeds

The length of time that embryos retain their viability, often referred to as their lifespan, varies enormously. Periods ranging from a few days to several thousand years have been reported. In general, it can be said that seed viability is governed by external

environmental factors to which the seed is exposed following maturation. Many seeds can be stored for months or even years under conditions of low moisture, low oxygen levels, and low temperatures. For other seeds, dry storage at ordinary levels of oxygen and temperature is necessary to maintain seed viability. Still other seeds appear to require storage under cool, moist conditions.

The longest life spans reported are for seeds that have been discovered accidentally in different natural situations. Seeds of the Indian lotus (*Nelumbo nucifera*) were found to be viable after being buried for about 1000 years in peat moss under a dry lake bed. Storage conditions were characterized by low oxygen levels. Several years ago seeds of a lupine (*Lupinus arcticus*) were found to have retained their viability for about 10,000 years. They had been buried in dry, frozen soil in the Arctic in the burrows of a small mammal. The ages of these seeds were estimated by geological and radiological (^{14}C-dating) methods.

Factors affecting Germination

The major environmental conditions necessary are access to water and air; a suitable range of temperatures; freedom from high concentrations of inorganic salts, poisons, and inhibitors; and, for some seeds, exposure to light. When dry seeds are placed in water or exposed to humid atmospheres, the first measurable process associated with germination is an increase in water content of the seed and its associated components (seed coat, endosperm or cotyledons, embryonic axis). The initial entrance of water to the seed occurs by imbibition.

The effects of the environment on seed germination are quite complex because of interactions and internal factors that modify germination patterns. A few of the major environmental factors are as follows:

Temperature: Seeds have been extensively used for studying the effect of temperature on physiological processes. Dry seeds are frequently able to withstand a broad range of temperatures, but after the germination process has been set in motion by the imbibition of water, most seeds appear to tolerate a much narrower range of temperature. The optimum temperature for seed germination varies according to species. The optimum temperature for most of the species ranges from 25 to 35° C. Generally, the germination is inhibited at very low and very high temperature. Temperature may interact with light and humidity affecting germination.

Water: The water is an important factor which controls and activates various enzymatic activities. It helps the seeds in imbibitions causing increase in osmotic effects.

Atmospheric Composition: Atmospheric composition also affects seed germination. The atmosphere consists of many gases like O_2, CO_2 and N_2 etc. Oxygen increases respiration. The percentage of O_2 for germination usually varies from 8 to 20. Excess of CO_2 concentration decreases germination in general but in few seeds like *Phleum pratense*

show increase in germination. Most of the pollutants like NO_2, SO_2, O_3, NH_3, H_2S, F and high concentration of ethylene inhibit germination.

Poisons and inhibitors: Different kinds of compounds are also known to affect seed germination. In some cases, the effects are quite specific for example, the action of hydrogen cyanide. Low concentrations of hydrogen cyanide or more specifically cyanide (CN), will poison and kill growing embryos. It is unlikely that cyanide will be found frequently in the immediate environment of a germinating seed. Some fruits, however, contain amygdalin, a glucoside composed of sugar and cyanide. If the fruit tissue is damaged, enzyme activity may hydrolyze the glucoside and release cyanide in concentrations great enough to kill the growing embryo. In other instances, the effect of a compound on seed germination may result from its influence on the moisture status of the immediate seed environment. Thus high salt concentrations (fertilizers or other inorganic salts) in contact with the seed may prevent the seed (by an osmotic effect) from obtaining enough water to initiate germination; or if the radical does manage to protrude through the seed coat, the embryonic tissue may become dehydrated and killed. Many soils contain high salt concentrations, either from irrigation or from soil minerals, which drastically inhibit seed germination.

Light: The seeds of most cultivated plants germinate equally well in light and dark. The light sensitive seeds are called photoblastic which may be of following three types:
(i) Positive photoblastic seeds: The seeds requiring single exposure of light for germination are called positive photoblastic seed, e.g., *Lactuca sativa*.
(ii) Negative photoblastic seeds: The seeds requiring complete darkness for germination are called negative photoblastic seeds
(iii) Non-photoblastic seeds: The seeds requiring either light or darkness for germination are called non-photoblastic seeds.
Photoblastic seeds can germinate in presence of light. The light induces phytochrome activity. Mayer (1986) proposed the involvement of calcium binding protein, the calmodulin, which stimulate the metabolic responses during germination.

Soil Conditions: The soil conditions which induce germination include water holding capacity, aeration of soil, mineral composition, soil texture, organic matter and pH of soil etc. Saline condition of soil inhibits germination. Other soil conditions also play important role in germination.

Seed Structure: Sometimes the seeds contain such structures which inhibit germination. These include hard and impermeable seed coat; reduced or rudimentary embryo and presence of germination inhibitors etc. These are discussed under seed dormancy.

Types of Seed

On the basis of number of cotyledons, seeds are primarily of two types.

- Monocotyledonous Seed

- Dicotyledonous Seed

Structure of a Monocotyledonous Seed

A Monocotyledonous seed has only one cotyledon. There is only one outer layering of the seed coat. A seed has the following parts:

1. **Seed Coat:** In the seed of cereals such as maize, the seed coat is membranous and generally fused with the fruit wall, called hull.
2. **Endosperm:** The endosperm is bulky and stores food. Generally, monocotyledonous seeds are endospermic but some as in orchids are non-endospermic.
3. **Aleurone layer:** The outer covering of endosperm separates the embryo by a proteinous layer called aleurone layer.
4. **Embryo:** The embryo is small and situated in a groove at one end of the endosperm. **Embryonal axis has p**lumule and radicle as the two ends.
5. **Scutellum:** This is one large and shield-shaped cotyledon.
6. **Coleoptile and coleorhiza:** The plumule and radicle are enclosed in sheaths called as coleoptile and coleorhiza respectively.

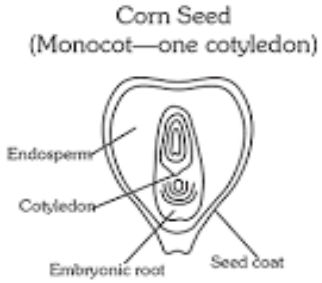

Fig. 5.1: Monocotyledonous seed

Structure of a Dicotyledonous Seed

Unlike monocotyledonous seed, a dicotyledonous seed has two cotyledons. It has the following parts:

- **Seed coat:** This is the outermost covering of a seed. The seed coat has two layers, the outer testa and the inner tegmen.
- **Hilum:** The hilum is a scar on the seed coat through which the developing seed was attached to the fruit.
- **Micropyle:** It is a small pore present above the hilum.
- **Embryo:** It consists of an embryonal axis and two cotyledons. Embryonal axis consists of **radicle and plumule** at the two ends.
- **Cotyledons:** These are often fleshy and full of reserve food materials.

- **Endosperm:** In some seeds such as castor, the endosperm is present while in plants such as bean, gram and pea, the endosperm is not present in the matured seed and such seeds are known as non-endospermic seeds.

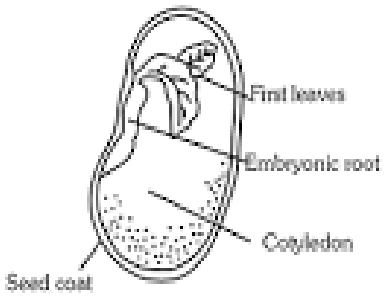

Fig.5.2: Dicotyledonous seed

On the basis of presence or absence of endosperm, seeds are categorised as endospermic and non endospermic seed respectively.

5.4 FRUIT DEVELOPMENT

Pollination is followed by fertilization and development of embryo. This is accompanied with many changes in the floral parts external to the embryo. There is a stimulation of cell division and cell enlargement in some of the tissues surrounding the embryo sac. Consequently, the appearance of the reproductive region changes almost entirely. These changes are the definite indication of the development of a fertile embryo within the ovary, and may be regarded as the reaction of the surrounding tissues to the growth of the embryo.

In most cases, it is the ovary which shows maximum response to the stimulus and develops into a fruit. In some plants, however, the ovules exert stimulatory effect on other floral parts including the floral axis itself. For instance, in strawberry (*Fragaria)* it is the receptacle supporting both the ovules and ovaries and in pineapple (*Ananas comosus*) the floral parts fuse with the inflorescence axis which develops into fruit. Thus the common definition of fruit is: Fruit is a **"transformed ovary"** but it resulted to contradictory concept of **'false'** or **'spurious'** fruits (*e.g.,* strawberries, pineapple which develop from parts other than Ovary). Natisch (1953) suggested a more appropriate definition of fruit as **"an aggregate of tissues which support the ovules of a plant and develops under the influence of these ovules".** Such a definition would also accommodate seedless fruits of certain varieties of grapes, banana, *etc.,* as in such fruits also ovules are originally present and at least trigger off the growth of the fruit.There is many fold increase in the size of the ovary during the formation of the fruit.

In brief the fruit is a ripened ovary. There is dual effect of fertilization on ovary. After syngamy ovule is changed into the seed. Side by side pollen tube triggers some mechanism

in the ovary wall which causes production of growth hormones, due to which the succulent parenchyma in ovary becomes much developed. Many acids, sugars and some other tasty substances are produced in these parenchymatous cells and ovary wall is changed into the **pericarp** after ripening. The pericarp may be thin or thick. Usually there are three layers in a thick pericarp. The outermost layer is known as the **epicarp,** middle one as the **mesocarp** and the inner most as the **endocarp.**

If fruit is developed from the ovary, the fruit is known as the true fruit. But sometimes some other floral parts participate in the formation of fruit, such fruits are known as **false fruits,** e.g., thalamus in apple is modified to form fruit.

The fruit is very useful for plants as it protects the seeds and helps in the dispersal of seeds.

Events leading to fruit development

The tissues which eventually form the fruit do not arise *de novo*, but they are already present in the flower. Sometimes the factors governing the initiation of the fruit tissue may be quite different from those stimulating the growth of other floral parts. For instance, low night temperature and short days can stimulate ovary initiation and growth preferentially as compared to the development of stamens and petals.

The first stage in the fruit development is rapid cell division in the ovarian tissue without much enlargement. This growth seems to be regulated by the developing ovules. If the ovules are removed or destroyed by parasites at an early stage before anthesis, there is marked reduction in the size of the ovary. However, the level of auxin in the ovules becomes very low at the time of anthesis. This explains why the growth of the ovary ceases when the flower opens, instead of continuing to form a full sized fruit.

During anthesis, the mode of growth of the ovary changes from cell division to cell enlargement. There are many evidences which suggest that this change is caused by auxins produced in the ovules. The shift in the growth phase is very gradual and the growth curve of the ovary is so smooth that from the point of view of the growth process, the distinction between cell multiplication and cell enlargement is unimportant.

Effect of developing seed on fruit development

Several experimental studies have shown that the changes in the ovary wall are greatly affected by the action of the developing ovules. It has been observed that if all the fertilized ovules are removed from an ovary, the development of fruit completely stops. In seedless grapes and some early varieties of peach and cherry, removal or destruction of embryos after a certain stage has no effect on fruit development as it proceeds even in the absence of viable seeds. In apple fruit, growth is strongly dependent upon seed development until the maturation of the embryo after which removal of seeds has a little effect on fruit growth.

In peaches, plums and cherries asymmetrical fruits are obtained when some of the fertilized ovules are removed but others left intact. Evidently this is due to localized growth around the ovules left intact. In strawberry, it was observed that if only one carpel is pollinated, an

area of fleshy tissue develops around that achene only. These observations indicate that only fertilized ovules are capable of inducing growth around them.

In seeds, endosperm is the site of auxin production. In rye grain 99.5 per cent of auxin is located in the endosperm especially in the aleurone layer. According to **Nitsch** *et al.***(1960)** the maximum concentration of auxin in the seed is at the time when the endosperm transformed from coenocytic to cellular condition.

Effect of growth regulators on fruit development

The observation that the fertilized ovule stimulates the surrounding tissues to divide and grow into a fruit led scientists to find out the nature of the stimulant. **Gustafson (1936)** was able to stimulate the fruit growth by applying synthetic auxins to unpollinated ovaries of *Lycopersicum, Petunia, Begonia, Nicotiana, Zephyranthes* etc. Similar results were obtained by **Wittwer (1943)** and **Luckwill (1948),** when they applied extracts of immature seeds to the unpollinated ovaries of tomato and sweet pepper. The growth factor present in the seed extract was also identified as auxin. Later, other plant hormones- cytokinins and gibberellins were found to have significant role in fruit development. Some fruits respond to gibberellins than to auxins. This difference is perhaps due to the natural concentration of one or another hormone that is present in the fruit.

Auxins and/or gibberellins stimulate the metabolic activity in the fruit tissues. Consequently, transport of water and other nutritive materials increases towards the fruit. This is also evident by the anatomical changes taking place in the stalk of the fruit. The concentration of reducing sugar and organic acids increases in the fruit tissue. The synthesis of starch is also augmented. These metabolic activities in the young developing fruit decrease the osmotic potential of the tissue. It is related to the increased absorption of water and growth and resulting in enlargement of cells.

Maturation of fruits involves many complex chemical and physiological changes. During maturation organic acids and starch is converted into free sugars and the activity of pectinase enzymes increases which soften and ultimately break down the cell wall. There is also loss of chlorophyll and elaboration of various pigments, mainly anthocyanins.

Several changes during maturation are induced by ethylene which is produced by the fruit itself. The ethylene produced by the fruit also stimulates other fruits to produce ethylene and mature faster. Ethylene induces a respiratory climate in maturing fruits. It increases the permeability of cell membrane which greatly facilitates the softening of the fruit and intermingling of respiratory metabolites and enzymes. Consequently, the respiratory process greatly speeds up.

Dehiscence of Fruits

Some fruits are indehiscent and some are dehiscent. The dehiscent fruits dehisce by the following methods and disperse their seeds.

i. **Sutural dehiscence:** These fruits dehisce by one suture as in follicle (*Calotropis)* or by two sutures as in legume (pea, bean) and seeds come out of the pericarp.

ii. **Porous dehiscence:** This type of dehiscence is usually found in capsule fruits. In this type of dehiscence, pores develop on the terminal end of the fruit through which seeds are dispersed by the wind e.g., *Papaver* (Poppy).

iii. **Transverse dehiscence:** This type of dehiscence is also found in capsule fruits. In this type of dehiscence pericarp dehisces transversely in two parts. The upper cap like portion is blown away by the wind and the seeds, kept in the lower part are dispersed by the wind e.g., *Celosia* (Cock's comb). *Eucalyptus. Psidium guyava* etc.

iv. **Loculicidal dehiscence**: In such type of dehiscence pericarp open through the middle of locules and the placenta is also divided e.g., *Gossypium* (cotton), *Hibiscus* (Lady's finger) etc.

v. **Septicidal dehiscence:** In this type of dehiscence septa is ruptured and locules get separated, pericarp is also separated in the form of valves e.g., Linseed, mustard, castor etc.

vi. **Septifragal dehiscence:** In this type of dehiscence placenta remains attached in the center of undivided fruit and pericarp's segments become separated. In this way actually this is the modification of septicidal and loculicidal dehiscence e.g. *Dhatura* (thorn apple) *Cedrela* (toon) etc.

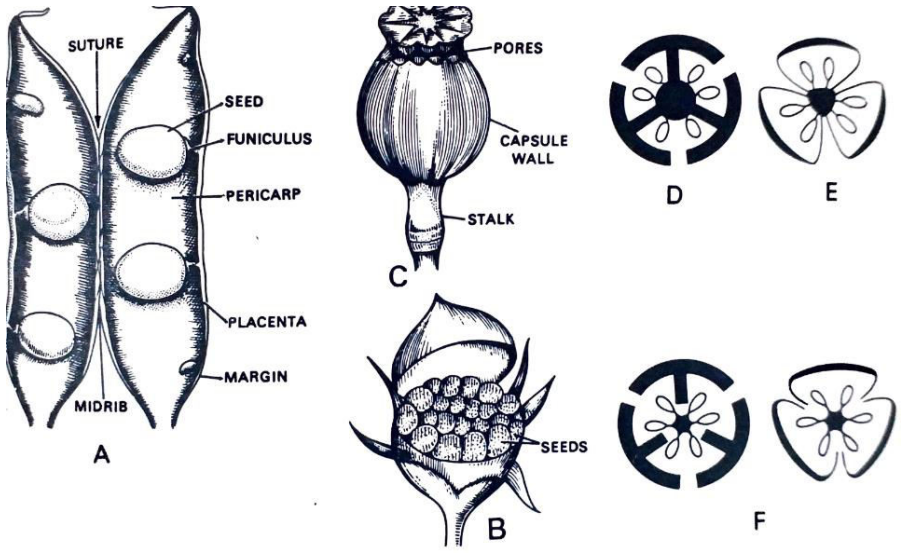

Fig.5.3: *Dehiscence of fruits. A. Sutural (bean), B. Transverse (Celosia), C. Porous (Poppy), D. Loculicidal (Bhindi), E. Septicidal (Castor), F. Septifragal (Datura)*

5.5 FRUIT TYPES

There are three main types of fruits:

1. **Simple fruits:** These fruits develop from monocarpellary ovary or multicarpellary syncarpous ovary.
2. **Aggregate fruits (Etaerio):** These fruits develop from the multicarpellary apocarpous ovary.

3. **Composite fruits (Multiple):** These fruits develop from the complete inflorescence.

Simple Fruits

These fruits can be divided into two types

1. **Dry fruits:** In these fruits pericarp is not distinguished into three layers. These are not fleshy.
2. **Succulent fruits (Fleshy fruits):** In these fruits, pericarp is distinguished into three layers: epicarp, mesocarp and endocarp. Mesocarp is fleshy or fibrous. These fruits are indehiscent, so seeds are separated after decay of the flesh.

Dry fruits

Dry fruits are divided into the following three groups.

A. **Dehiscent fruits (Capsular fruits):** In these fruits the pericarp is ruptured after ripening and seeds are dispersed.
B. **Indehiscent fruits (Achenial fruits):** These fruits do not dehisce after ripening and seeds remain inside the pericarp.
C. **Schizocarpic fruits (Splitting):** These fruits, after ripening are divided into one seeded segments or mericarp, but these mericarps do not rupture further. In this way this group of fruits is intermediate between dehiscent and indehiscent fruits.

A. Dehiscent Fruits (or capsular fruits)

These fruits can be divided into following five groups:

i. **Legume or pods:** These fruits develop from monocarpellary superior ovary. Ovary has marginal placentation, one locule and many ovules. Mature fruits have one suture on each side through which these fruits dehisce (open) e.g., Pea, Bean etc.

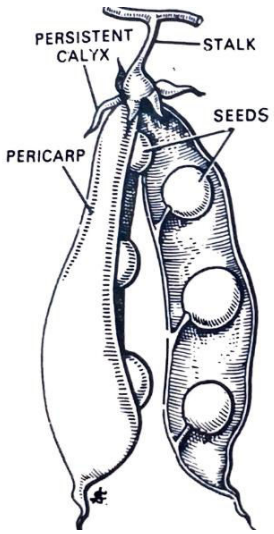

Fig.5 4: Legume of pea

ii. **Follicle:** These fruits have only one suture. Other characters of these fruits are similar to the legume fruits. These are found usually in pairs or more than two in group e.g., larkspur, *Calotropis, Michelia,* penwinkle.

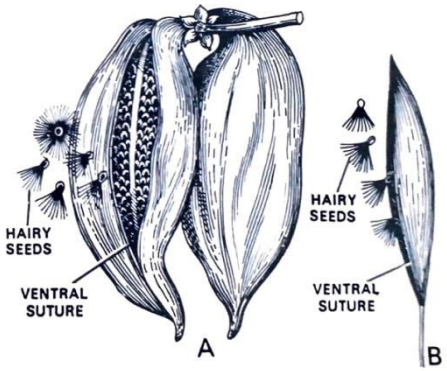

Fig.5.5: A. Follicle of Madar B. Follicle of milkweed with liberated seeds

iii. **Siliqua**: This fruit develops from bicarpellary, syncarpous, superior ovary. Ovary has parietal placentation. Ovary, in the beginning is unilocular but later due to development of false septum becomes bilocular. Each locule has many seeds. On maturation pericarp ruptures in two valves. Dehiscence starts from lower part and proceeds upward. False septum remains as such in the middle of parietal placentation and it is jointly known as replum. Seeds are dispersed by wind. This type of fruit is the characteristic feature of family Brassicaceae e.g. *Brassica.*

iv. **Silicula:** It resembles the siliqua but it's breadth and length are equal. It is wide and flat e.g., *Capsella* (Shepherd's purse).

v. **Capsule:** These fruits develop from multicarpellary, syncarpous, superior ovary and sometimes from inferior ovary. These are multilocular and many seeded fruits and dehisce by various methods e.g. *Hibiscus esculentus* (Lady's finger).

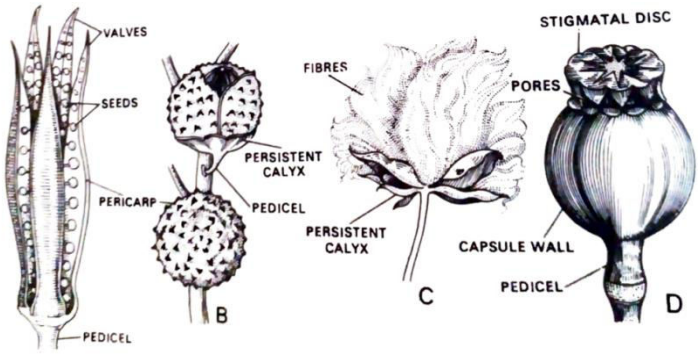

Fig.5.6: Capsular fruits A. Bhindi B. Datura C. Cotton D. Poppy

B. Indehiscent or Achenial Fruits

i. **Achene:** These fruits develop from monocarpellary superior ovary. These fruits are unilocular and one seeded and pericarp is free from the seed coat.

Most of the achenes are found as etaerios because these usually develop from multicarpellary apocarpous ovary e.g. *Clematis, Narvelia.*

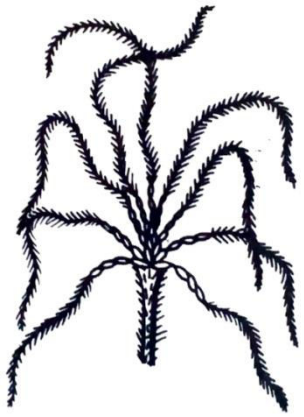

*Fig.**5.7:** Achene (Etaerio)of Narvelia*

ii. **Caryopsis**: These fruits develop from monocarpellary, superior ovary. These fruits are unilocular and one-seeded. Pericarp of these fruits is fused with the seed coat. These fruits are present in Family Poaceae e.g., wheat, rice, maize.

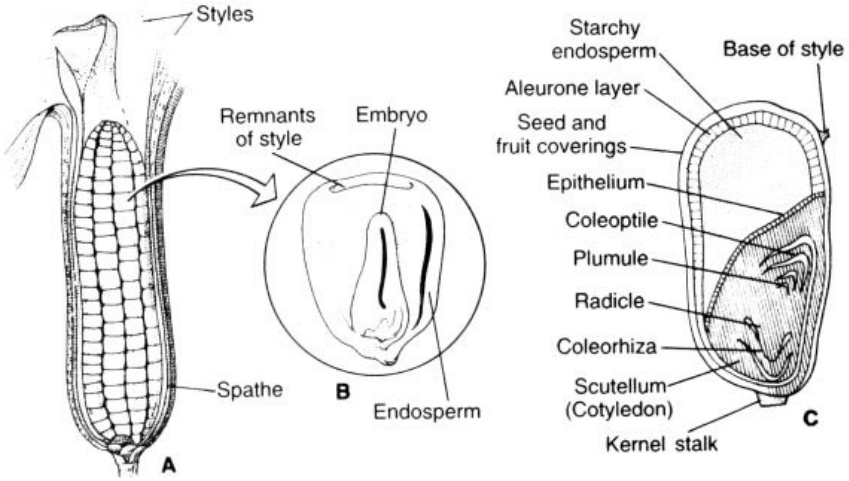

Fig. 5.8: Maize. A. Cob of maize B. Single grain (Caryopsis) of maize C. L.S. of maize grain

iii. **Cypsella:** These fruits develop from bicarpellary, syncarpous, inferior ovary. These are unilocular and one seeded. Pericarp is free from the seed coat. Hairy calyx attached with the fruit is present in these fruits, which is known as pappus.

Pappus helps in parachute mechanism for fruit dispersal by wind. It is usually present in family Asteraceae e.g., sunflower, *Tagetus, Cosmos, Taraxacum.*

iv. **Nut:** These fruits are also unilocular and one seeded. These develop from bi or multicarpellary, syncarpous, superior ovary. Pericarp in these fruits becomes hard and stony e.g., *Anacardium* (Cashew nut), Litchi, Oak, *Trapa.* In litchi fleshy aril is edible.

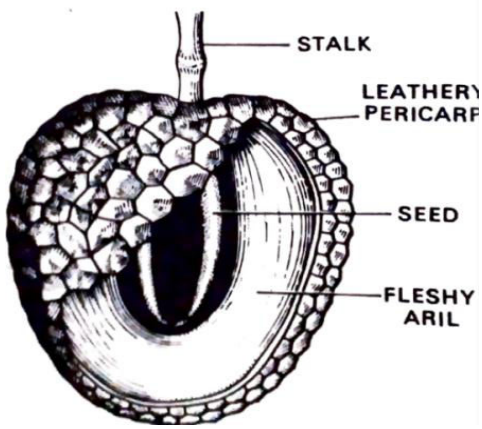

Fig.5.9: *Nut of Litchi*

v. **Samara:** These fruits are one seeded. Pericarp of these fruits become flat like wings e.g., *Holoptelea indica* (Chilbil).

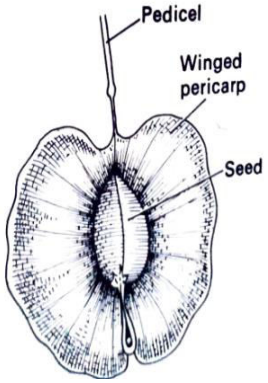

Fig.5.10: Samara of Holoptelea

C. Schizocarpic or splitting fruits.

These fruits are classified as follows:

i. **Lomentum:** These fruits develop from monocarpellary superior ovary. Actually, these fruits are the modifications of legumes. These are bisutural fruits which are constricted or divided in one seeded mericarps e.g. *Mimosa pudica,* ground nut, Indian laburnum, *Acacia, Tamarindus.*

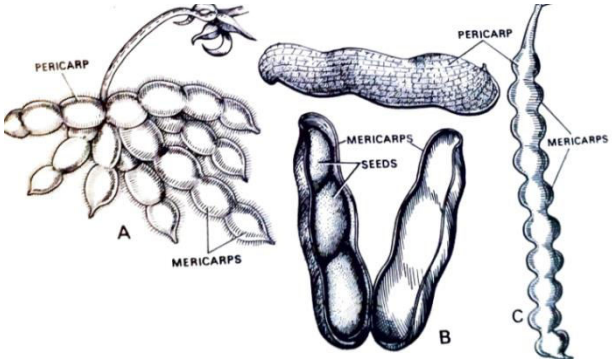

Fig.5.11: Lomentum. A.Mimosa pudica B. Ground nut C. Acacia

ii. **Cremocarp**: These fruits are bilocular and two seeded and develop from bicarpellary, syncarpous, inferior ovary. On maturation these divide along with carpophores (which is the apically grown part of thalamus) into two mericarps, each mericarp has one seed. These fruits are present in family Apiaceae (Umbelliferae) e.g. Coriander, Carrot etc.

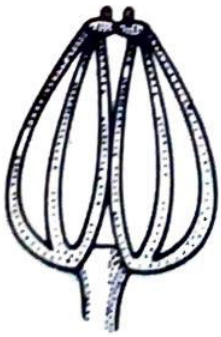

Fig.5.12: Cremocarp of Coriander

iii. **Regma**: These fruits develop from multicarpellary pistil and on maturation (after splitting) these divide into as many parts as the number of carpels. Each part of known as coccus. Each coccus is one seeded. Castor has three cocci and *Geranium* has five cocci, e.g., castor.

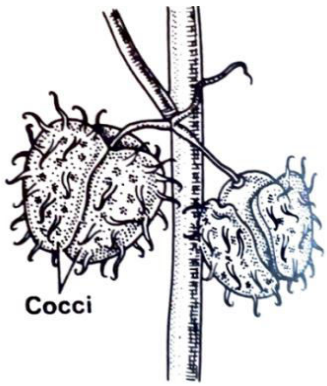

Fig.5.13: Regma of castor

iv. Carcerulus: These fruits develop from bicarpellary, syncarpous superior ovary. Due to formation of a false septum, four one-seeded locules are formed. Four one-seeded mericarps of this type are present in family Lamiaceae (Labiatae) e.g., *Ocimum.* In Hollyhock of family Malvaceae and *Abutilon,* these fruits develop from multicarpellary pistil. Hence, number of mericarps in these fruits is more.

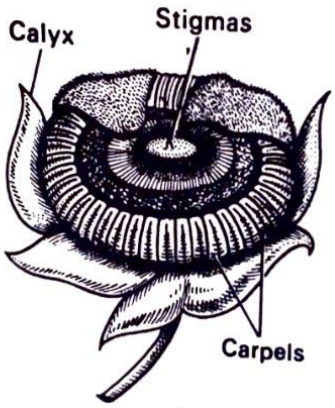

Fig.5.14: Carcerulus of Hollyhock

v. **Double Samara:** These fruits develop from bicarpellary, syncarpous, superior ovary. Pericarp in these fruits develops in two wings. On maturation, this is divided in two one seeded parts e.g., *Acer*

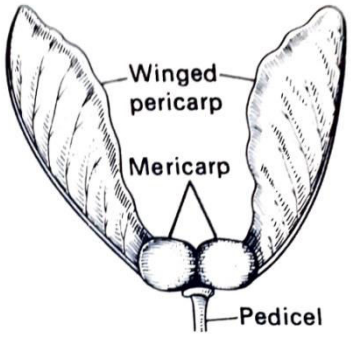

Fig. 5.15: Double samara of Acer

2. Succulent or Fleshy Fruits

Succulent fruits can be divided into the following five groups :

i. **Drupe:** These fruits develop from mono or multicarpellary, syncarpous, superior ovary. These fruits are uni or multilocular and one or many seeded. Epicarp of these fruits forms the skin of fruits. Mesocarp is fleshy or fibrous and endocarp is hard and stony e.g., mango, cherry, coconut, almond, walnut and plum.

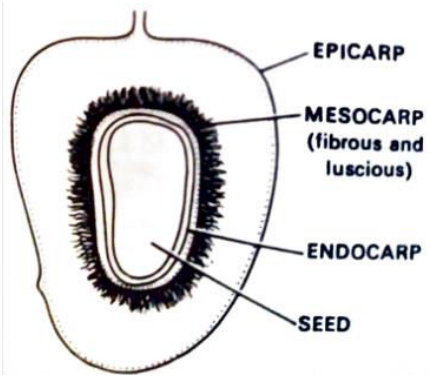

Fig.5.16: Drupe of mango

ii. **Berry or Bacca:** These fruits develop from mono or multicarpellary syncarpous ovary. Ovary is superior and sometimes inferior. Placentation is axile or parietal.
Epicarp in these fruits makes the rind of fruit. Mesocarp is fleshy and endocarp is thin like a membrane e.g. tomato, brinjal, *Psidium gujava*, banana, grape, date palm, papaya, cheeku, arecanut (supari).

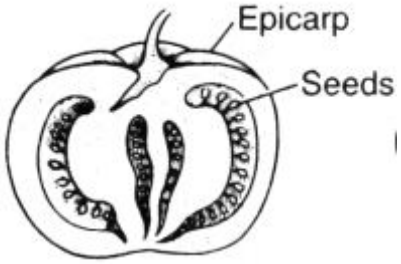

Fig.5.17: Berry or Bacca of tomato

iii. **Pepo:** These fruits resemble berries, but these develop from inferior ovary with parietal placentation. These fruits are full of swollen placenta and are many seeded. Epicarp makes a hard rind. This type of fruits is specially found in family Cucurbitaceae e.g., bottle gourd, cucumber, muskmelon.

iv. **Hesperidium:** These fruits develop from multicarpellary, syncarpous, superior ovary. Ovary has axile Placentation. These are multilocular. Many oil glands are present in the epicarp. Mesocarp is in the form of white, fibrous part fused with epicarp. Membranous endocarp projects inwards forming distinct chambers. Many unicellular juicy hairs are present on the inner side of endocarp. These are edible parts e.g., Orange. Lemon

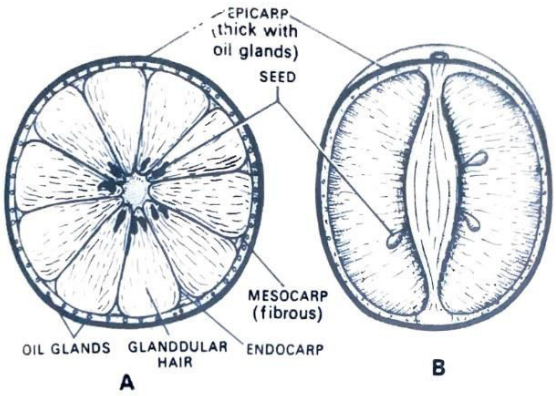

Fig.5.18: Hesperidium of Citrus. A. T.S., B. L.S.

v. **Pome:** This fruit develops from the fleshy thalamus of syncarpous, inferior ovary so it is a false fruit. True fruit remains inside the swollen thalamus. Wall of the ovary is thin like a paper and seeds develop inside the locules. Fleshy swollen thalamus of these fruits is the edible part e.g., apple, pear.

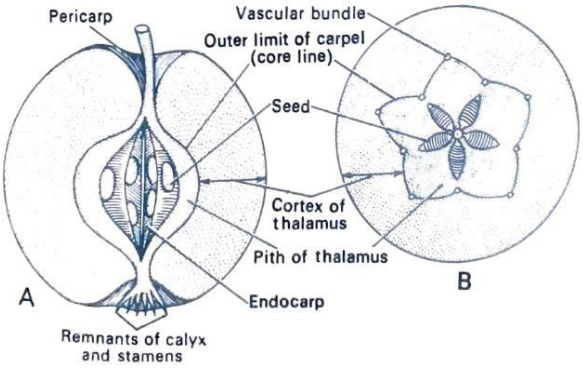

Fig. 5.19: Pome of apple. A. L.S., B. T.S.

vi. **Balausta:** This fruit develops from multilocular, syncarpous, inferior ovary. Testa is fleshy and forms edible part of the fruit. Tegmen is hard. Seeds are irregularly arranged in the fruit. Pericarp is rough and leathery. The fruit has persistant calyx, e.g. pomegranate

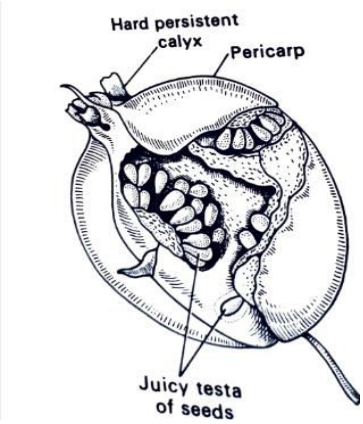

Fig.5.20: Balausta of pomegranate

vii. **Amphisarca:** This fruit develops from multicarpellary, syncarpous multilocular superior ovary. Epicarp is hard. Mesocarp and endocarp are fleshy on which are scattered many seeds. Mesocarp, endocarp and swollen placenta are eaten i.e., *Agave marmelos (*wood apple).

II. Aggregate Fruits

Aggregate fruits are the group of fruitlets which develop from the multicapellary, apocarpous ovary. Such type of aggregate fruits, develop from the single flower is known as etaerio. These fruits make an aggregate of fruitlets. This aggregation is known as etaerio of fruitlets e.g., if fruit is achene, aggregate of fruit will be etaerio of achenes.

Aggregate fruits are of the following types.

i. **Etaerio of follicles:** In such fruits, each free (separate) carpel develops into a fruitlet which is known as follicle. Many follicles (fruitlets) make an etaerio which are arranged on the enlarged thalamus in a bunch (group) e.g., etaerio developed from two follicles, *Calotropis* and *Catharanthus.* Etaerio developed from many follicles – *Michelia.*

Fig.5.21: Etaerio of follicles in Michelia

ii. **Etaerio of berries:** It is an aggregate fruit of small berries e.g., in custard apple, many berries develop on all side of fleshy thalamus. Apical parts of berries fuse with each other and make a common rind. E.g., *Polyalthea, Anona squamosa* (Hindi-Sharifa)

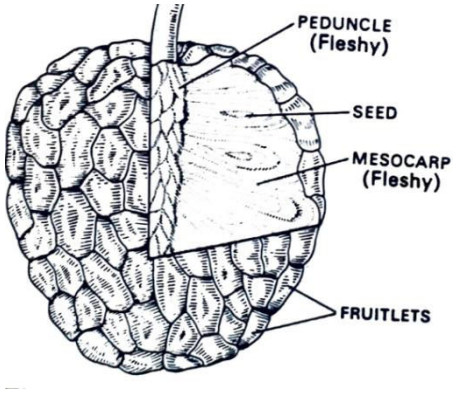

Fig.5.22: Eterio of berries in Anona

iii. **Etaerlo of achenes:** Each fruitlet is an achene e.g. *Narvella, Clematis.* Achenes are hairy. In rose, many achenes are present on a saucer (cup) like thalamus. In lotus, thalamus becomes spongy and some achenes are embedded in it. The thalamus is fleshy and becomes red on maturation and is the edible part of *Fragaria* (strawberry).

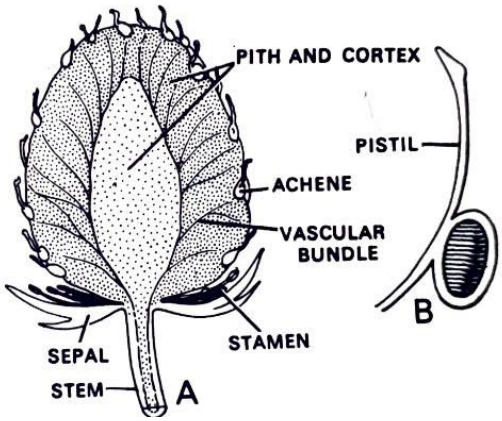

Fig.5.23: Eterio of achenes of strawberry

iv. **Elaerio of drupes:** In this type of fruit, many small drupes, develop from different carpels, are arranged collectively (in groups) on the fleshy thalamus e.g. *Rubus idaeus*.

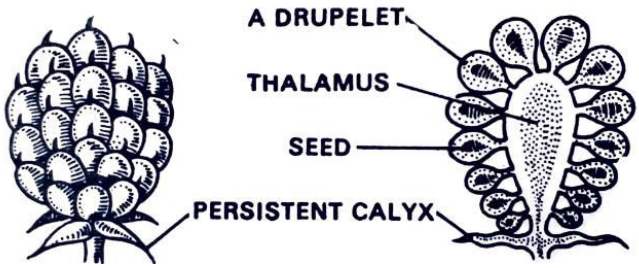

Fig.5.24: A. Etaerio of drupes in Rubus. B. L.S. of the etaerio

Composite Fruits (Multiple Fruits)

These fruits develop from the complete inflorescence. These are known as fructescence. These are of two types.

i. **Sorosis:** These fruits develop from spike, spadix or catkin inflorescence. In jack fruit (Hindi-Kathal) (*Artocarpus integrifolia)* pistillate flowers develop very close to each other around the rachis. Bracts, perianth and seeds become simple and are used for eating. Stigmas fuse with each other to make rough and spiny rind. In pineapple (*Ananas sativus)* too, rachis, bracts and perianth are the edible parts. In mulberry fleshy perianth present around the dry achenes is eaten.

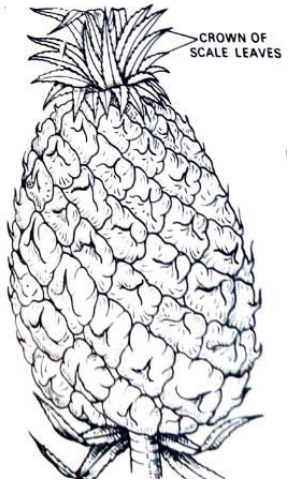

Fig.5.25: Sorosis of pineapple

ii. **Syconus:** This type of fruit develops from hypanthodium inflorescence. Receptacle becomes hollow having a pore surrounded by small scales. Inside the receptacle are present unisexual flowers on the wall. Staminate flowers are present near the pore and normal pistillate as well as gall pistillate flowers are present on the lower side. Larvae of insects

live in gall flowers. Receptacle becomes fleshy and many achenes develop from the pistillate flowers. Rachis (Fleshy receptacle) of these fruits is edible part.

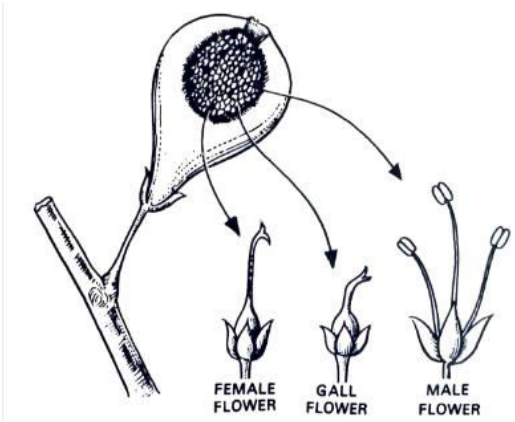

Fig.5.26: Syconus. L.S. of fruit and three types of flowers

5.6 SUMMARY

A seed consists of a plant embryo surrounded by a seed coat. Seeds may store food within or outside the embryo. In most dicotyledonous plants such as bean, food is stored in the two cotyledons. Cotyledons may also serve as absorbing and, later, as photosynthesizing organs. Food may be stored in an endosperm rather than in the cotyledons. The first step in germination is the imbibition of water. This water facilitates the activation of enzymes involved in digesting stored food, which is converted to energy for growth. In germination, the cotyledons may be elevated above the ground (epigeal), sometimes becoming photosynthetically active, or they may remain below the ground (hypogeal). Mature seeds may be dormant and, depending on the species and the immediate environment may remain viable and dormant for a few months to years. Dormancy is usually broken by providing the seed with moisture, oxygen, and a favourable temperature. Other factors, such as light, the removal of chemical inhibitors, or the treatment of the seed coat may be required in some instances.

A fruit is a ripened ovary plus other closely associated floral parts. There are three different kinds of fruits, classified on the basis of the number of ovaries and flowers involved in their formation i.e. simple fruits, derived from a single ovary; aggregate fruits, derived from a number of ovaries belonging to a single flower and on a single receptacle and multiple fruits, derived from a number of ovaries of several flowers more or less grown together into one mass. The role of the fruit is to help in the dispersal of the seeds within and to avoid or discriminate herbivores from eating the seeds without dispersing them. Fruits protect seeds from herbivores by the timing of their ripening, their hardness, and their chemical composition. Fruits may contain secondary compounds that deter feeding by all but the most metabolically specialized herbivores. Some fruits prevent or limit dispersal, thus

ensuring that the site occupied by the parent plant will be occupied by its offspring well into future growing seasons.

5.7 GLOSSARY

Achene: A simple, one-seeded fruit in which the seed is attached to the ovary wall at only one point, such as the "seed" on the surface of a strawberry.
Aggregate fruit: Fruit made up of two or more carpels from a single flower, plus the stem axis (Example – blackberry).
Berry: It consisting of one or more carpels with one or more seeds, the ovary wall fleshy
Caruncle: Arilloid (type of outgrowth on the seed) occurring near the micropyle.
Caryopsis or grain: A one-seeded fruit in which the seed is firmly attached to the fruit at all possible points
Cotyledons: In seeds, the embryonic leaves, which in many species have absorbed the entire or the major part of the nucellus and endosperm, hence becoming the principal nutrient storage tissue.
Dehiscent fruits: Fruits which dehisce or split open when fully mature
Drupe: It is a stone fruit, derived from a single carpel and containing (usually) one seed.
Drupelet: A small drupe, the unit making up the fruit (Examples- raspberry, blackberry).
Dry Fruits: Pericarp is dry at maturity
Fleshy Fruits: Pericarp fleshy at maturity
Follicle: Fruit composed of one carpel and splitting along a single suture
Hard seed: Seeds with hard, impermeable seed-coat that prevents imbibition. The hard seed-coat also serves as protection against physical damage.
Hesperidium: It is a specialized berry with a leathery rind
Hilum: In angiosperms, scar on the seed-coat left by the funiculus. Gymnosperms have no funiculus, but a hilum-like scar may appear where the seed has been attached to the megasporophyll.
Legume: Fruits composed of a single carpel and splitting along two sutures.
Loment: Fruits having several seeds, breaking into one-seeded segments at maturity
Mesocarp: The middle of three layers of the fruit wall.
Nut: A hard, one-seeded fruit, generally formed from a compound ovary, with the pericarp hard throughout
Pepo (an accessory fruit), a berry with a hard rind, the receptacle partially or completely enclosing the ovary
Pericarp: The fruit wall, consisting of three distinct layers: the exocarp, the mesocarp, and the endocarp.
Plumule: The embryonic shoot derived from the epicotyl. In dicotyledons situated between the cotyledons.
Pome (an accessory fruit), derived from several carpels, receptacle and outer portion of pericarp fleshy, inner portion of pericarp papery or cartilaginous forming a core

Radicl: The embryonic root, i.e. the part of the seed embryo that develops into the primary root. In seeds, the radicle is always facing the micropyle.
Raphe: Ridge formed on the seed-coat if the funiculus is fused with the integument in part of its length in anatropous or campylotropous ovules.
Samara: A one- or two-seeded fruit with the pericarp bearing a wing like outgrowth. A modified achene
Scarification: Disruption of hard seed-coats, usually by mechanical abrasion or by brief chemical treatment in a strong acid, to increase their permeability to water and gases, or to lower their mechanical resistance.
Schizocarp: Fruit consisting of two carpels which at maturity separate along the midline into two one-seeded halves, each of which is indehiscent
Seed Longevity: The period of time seed will maintain viability in storage under a given set of storage conditions. Often used equivalent to storability.
Seed: The mature ovule, consisting of an embryo, and possible nutritive tissue, enclosed by the protective seed-coat derived from the integuments.
Seed-coat: Protective outer layer(s) on a seed derived from the integuments. When two layers of the seed-coat are distinguishable, the terms testa for the outer coat and tegmen for the inner is often used.
Simple fruit: Fruit developing from one carpel. Example – acorn, bean.
Tegmen: Part of the seed-coat produced by the inner integument, usually thin and delicate.
Temperate fruit: A fruit plant that requires a cool period and is deciduous. Examples - apple, pear, and peach.
Testa: The seed-coat; by some authors it refers only to that part of the seed-coat that is produced by the outer integument.
Viable seed: A seed which can germinate under favourable conditions provided that any dormancy that may be present is removed.

5.8 SELF ASSESSMENT QUESTIONS

5.8.1 Multiple choice Questions:

1. Which of the following fruits have a missing endocarp and the seeds are found scattered in the mesocarp?
a.Berry b. Drupe
c. Pome d. Caryopsis

2. Which of the following fruits undergo parthenocarpy naturally?
a. Apple b. Mango
c. Banana d. Plums

3. During seed germination, seed coat ruptures due to
a. Differentiation of cotyledons b. Massive glycolysis in endosperm and cotyledons
c. Sudden increase in cell division d. Massive imbibitions of water

4. Seed develops from
a. Ovary b. Embryo
c. Ovule d. Embryo sac

5. An albuminous seed showing hypogeal germination is
a. castor b. Bean
c. Gram d. Maize

6. Proteinaceous part of maize endosperm is
a. Apophysis b. scutellum
c. Aleurone layer d. Peripheral layer

7. Vivipary is
a. Seed germination with subterranean cotyledons b. Seed germination with epiterranean cotyledons
c. Fruit development without pollination d. seed germination inside the fruit while attached to the plant

8. A gas required for germination of pea seed is
a. Nitrogen b. Oxygen
c. Hydrogen d. Water vapours

9. Seed dormancy allows the plants to
a. Overcome unfavourable climate conditions b. Develop healthy seeds
c. Reduce viability d. Prevent deterioration of seeds

10. Among the following which compound can induce seed dormancy?
a. ABA b. Potassium nitrate
c. Gibberelllins d. Ethylene

11. Protective covering over radical during seed germination is
a. Suspensor b. coleorhiza
c. Epithelium d. Coleoptile

12. Fruit is a
a. Post fertilization product of pistil b.Product of flower

c. Body having seeds d. Product of ovary

13. A true fruit is
a. Developed ovule b. Developed ovary
Fertilized and developed ovary d. Fertilized and developed ovule

14. An accessory fruit is the one which develops from
a. An ovary b. Ovary and thalamus
c. Unfertilized ovary d. Inflorescence

15. Parthenocarpy is a fruit
a. Formed from superior ovary b. Formed from inferior ovary
c. Consisting of ripened ovary and thalamus d.Which does not possess seeds

16. Indehiscent single seeded dry fruits are
a. Composite b. Aggregate
c. Achenial d. Schizocarpic

17. One seeded winged fruit is
a. Cypsela b.Samara
c. Achene d. Nut

18. Fruit of Litchi is
a. Caryopsis b. Nut
c. Berry d. Drupe

19. The fruit of fig is
a. Syconus b. Sorosis
c. Berry d. Balausta

20. The edible portion of tomato is
a. Epicarp b. Placenta and pericarp
c. Mesocarp only d. Thalamus

5.8.1 Answers Key:

1. (a) 2. (c) 3. (d) 4. (c) 5. (d) 6. (c) 7. (d) 8. (b) 9. (b) 10. (d) 11. (b) 12. (d) 13. (c) 14. (b) 15. (d) 16. (c) 17. (b) 18. (b) 19. (a) 20. (b)

5.8.2 Short Answer Questions

1. Which parts of the following fruits are edible: Litchi, mango, papaya, strawberry, pine apple.

2. Write down short note on the following : (i) Schizocarpic fruits (ii) Sorosis (iii) Syconus
3. Classify the following fruits; (a) Almond (b) Date palm (c) Fig (d) Custard apple
4. Why is the pome fruit known as a false fruit.
5. Which of the following is a false fruit and why?

a. Wheat b. Cucumber c. Apple d. Jack fruit

6. Write the different parts of the seed.
7. What are essential conditions for a seed to grow into plant?
8. What are cotyledons?

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5.11 TERMINAL QUESTIONS

1. Describe types of simple dry fruits.
2. What are drupes? Give its different types.
3. What are berries? Give structure of berries of different fruits.
4. What are aggregate fruits? Give examples.
5. Describe the processes that occur during germination.
6. What is seed dormancy? Why is it an important process?
7. What are the differences between simple, aggregate, and multiple fruits?
8. Mention two main differences between drupe fruit and schizocarpic fruit. Explain any four types of schizocarpic fruits with suitable diagrams and example
9. Describe the following fruits and mention their edible parts. Apple, coconut, mulberry and orange.
10. Differentiate between the following. Give examples also.
    (a) Drupe and berry
    (b) Follicle and pod
    (c) Etaerio and composite fruits
    (d) Achene and Cypsella.
11. Differentiate between:
    (a) Epicotyl and hypocotyl
    (b) Coleoptile and coleorrhiza
    (c) Perisperm and pericarp
12. Write down the conditions necessary for seed germination.

UNIT-6: SIGNIFICANCE OF HORMONES AND ENVIRONMENTAL FACTORS IN SEED GERMINATION

6.1 OBJECTIVES

After reading this unit, students will be able:

- To understand the importance of Seeds.
- To understand the concept of Seed germination.
- To understand the role of different hormones in the process of seed germination.
- To understand the effects of environmental factors in the process of seed germination.

6.2 INTRODUCTION

Most of the plants at some phase of their life cycle raise a spreading unit in large quantity which can endure severe climatic state and is capable of persisting without visible morphological alteration over significant phase. Moreover, the dispersal unit from a system assures the distribution and existence of species under conditions which could generally destroy life forms. On reappearance of favorable state, active vegetative development continues through the stimulation of germination.

Seed germination is one the crucial phases in the life cycle of a plant which is marked by hormones, environmental factors and dormancy. It is a mechanism in which morphological and physiological variations end in the initiation of embryo. Before germination, seed absorbs water leading to the development and elongation of embryo. When the radicle emerges from the seed coat, the process of seed germination is completed. As the whole plant exists in the seed hence it can be said that seed is an important structure. This chapter provides the complete information regarding the biology of seeds which are characteristic of Spermatophytes (*i.e.* Angiosperms and Gymnosperms).

Though you have studied in previous chapter but just to remind, a mature seed is having three main parts:

1. Seed coat
2. Embryo
3. Endosperm

Seed coat: Outermost, protective covering of the seed is referred as seed coat which progresses from integuments of ovule. Those seeds which grow from bitegmic ovules, there occurs two separate layers in seed coat. The outer layer which is thick, rigid and leathery grows from the outer integument is referred as testa and the thin and membranous inner layer grows from the inner integument is tegmen. Those seeds which grow from

unitegmic ovule contain only a single layer in seed coat which is usually protective in function.

Embryo: It is the most significant portion of the seed which symbolizes tiny future plant. Embryo grows from fertilized egg *i.e.* zygote. The embryo is having a main axis which is referred as tigellum to which one or two seed leaves which are also called as cotyledons are attached depending upon the seed whether it is monocot or dicot. The measure of embryonal axis beneath the point of attachment of cotyledons is referred as hypocotyl which gives rise to radical *i.e.,* root. Likewise, the measure of embryonal axis above the point of attachment of cotyledons is referred as epicotyl which gives rises to plumule i.e., shoot.

Endosperm: It is the nourishing tissue which may be either present or absent in the seeds. On the basis of presence or absence of the endosperm in seeds, the seeds in angiosperms are broadly classified into two categories:

a. Endospermic seed **b. Non endospermic seed**

a. Endospermic seed: In this kind of seeds, food is deposited in endosperm. This is the reason they are referred as endospermic seeds or albuminous seeds. The endospermic tissue present in these seeds consumed during the germination of seeds and their cotyledons are skinny and membranous. Examples of endospermic monocot seeds are maize and wheat whereas endospermic dicot seeds are in castor and papaya.

b. Non endospermic seed: This kind of seed does not contain endosperm at maturity and thus are referred as non-endospermic or exalbuminous seeds. The endospermic tissue is consumed during the expansion of embryo. As the absorbed food materials from the endosperm are deposited in cotyledons thus it becomes huge and fleshy. Example- *Capsella.*

6.3 SIGNIFICANCE OF HORMONES

Plant hormones are physiological intercellular messengers which a play vital role in regulating overall cycle of a plant. They are produced in response to environmental components viz. abundance of nutrients, water scarcity, physical or chemical stress and elevated temperature. Every feature of plant growth and development is under hormonal regulation to some extent. A particular hormone can standardize distinct range of cellular and progressive processes comprising growth, germination, fruiting, flowering, foliage and death.

There are different classes of plant hormones *viz*. Auxin, cytokinin, gibberelic acid, abscisic acid, ethylene and brassinosteroids which are well known to regulate life cycle of a plant.

6.3.1 Auxin

This hormone was discovered by Went in 1928 which plays a fundamental role in controlling the cell cycle, growth and development, root initiation, flower initiation, formation of vascular tissues, parthenocarpy etc. Auxin by itself is not a vital hormone for the germination of seeds but the investigation regarding representation of auxin related genes, auxin is synthesized in seed radicle tip during as well as after the process of germination.

Among various auxins, Indole-3 acetic acid (IAA) is capable of influencing the germination of seeds by marking the action of enzymes *viz*. in germinating pea seeds, the activity of glyoxylase I is controlled by IAA leading to higher proportion of cell growth and development.

6.3.2 Cytokinin

This hormone was isolated from Herring fish sperms DNA, proven to control broad array of plant activities *viz.* cell division and enlargement, secondary growth in plants by the formation of interfascicular cambium, delay in the process of senescence, breaking seed dormancy, induce stomatal opening and seed germination. Cytokinin is also able of augmenting seed germination by the mitigation of stress *viz.* heavy metal stress, oxidative stress, salinity stress and water deficit stress. Important cytokinins are kinetin (Kn), Benzylamino purine (BAP or BA) etc.

6.3.3 Gibberellic acid

This hormone is found in all groups of plants and was first separated from the metabolite products of rice pathogenic fungi *i.e., Gibberella fujikuroi*. It is a diterpenoid and controls the plant growth and generally used in agriculture. It has been widely used for the purpose of germination and thus gibberellic acid is also referred as “enhancers of seed germination”. This hormone augments seed germination by impeding ABA activity triggered by the activity of catabolizing enzymes and hindering the related biosynthetic pathways which also lower abscisic acid quantity.

Gibberelic acid also accelerates the production of hydrolases especially α- amyalases ensuring the germination of seeds. Additionally, it is also known to stimulate an array of genes which play vital role in the production of amylases *viz.* α- amyalase, β- glucanase and protease.

6.3.4 Abscisic acid

Abscisic acid is also referred as "Stress hormone" obtained from the mature cotton fruits by Addicott and Okhuma in 1963 and was given the name "Abscisin II".

It hinders the germination of seeds by slowing down the development of radicle and drooping of endosperm along with the increased illustration of transcription factors which negatively influence the process of seed germination.

6.3.5 Ethylene

Ethylene is a gaseous hormone distinguished as a natural plant growth regulator by Goeschl. Competed with other plant hormones, ethylene has the capacity to make quiescent seeds germinate by controlling the illustration of cysteine protenase gene and its protein composite *i.e.* proteasome.

6.3.6 Brassinosteroids

They characterize the class of plant hormones analogous to steroid hormones in other individuals and were first isolated from the pollens of *Brassica napus*. This hormone has varied scope in terms of plant growth and development comprising seed germination, flower induction, cell growth and reproductive growth. They improve the germination rate of seeds by regulating the inhibitory effects of abscisic acid on seed germination.

In a nut shell, gibberelic acid, brassinosteroids, and ethylene improve the capacity of embryo to emerge from the seed by splitting the endosperm and antagonistically relating with abscisic acid. These three hormones increase the germination of seeds via specific signaling pathways.

6.4. SIGNIFICANCE OF ENVIRONMENTAL FACTORS

Ecophysiological studies have suggested that effective formation of plant species is frequently determined by the timing of germination rising from the seed reaction to

environmental signals. Seeds act in response to a sequence of varied environmental features *viz*. light, temperature, salinity stress, soil environment and depth of implanting.

6.4.1 Light

It is one of the environmental factors that may control germination of seeds and initial formation of seedlings. All plant species differ in their light requisite for the germination of seeds. Some seeds need light for the germination while others do not require light. Seeds depend on light to supply information because the energy is provided by the seed reserves. The light triggers phytochrome photoreceptor in the seeds which play active role in regulating period of germination and thus become critical measure of the evolutionary approach to recover restricted dormancy to look after seedlings from environmental extremity.

As a general rule, small seeded species are more susceptible to light for germination as compared to the species with larger seeds.

6.4.2 Temperature

Different plant species have varied temperature scale above which their seeds will germinate. In general, alternating temperature treatments can be more operative in the promotion of germination as compared to steady temperature (*i.e*. very high and low temperature). This sensitivity fluctuates among species. For the existence of species, it is essential that seeds should germinate only when the environmental aspects are likely to be suitable for growth of the seedling (temperature mark germination of seeds by controlling activity of enzymes drawn in the progression of germination and by promoting or delaying the synthesis of hormones that affect seed dormancy).

6.4.3 Salinity stress

Salinity stress lead to drop the rate of seed germination as it effects seed germination by generating peripheral osmotic potential that hinders uptake of water leading to toxic ion effects. Additionally, sodium ions can modify soil organization and its fertility by restoring calcium and magnesium ions in the process of anion exchange thus leading to nutrient and water strain desirable for the process of seed germination.

6.4.4 Soil environment

Soil ought to be properly hydrated (as insufficient water will inhibit metabolism whereas excess amount of water literally drown the seeds which die of anoxia), should be having proper oxygen levels, proper size of soil particles (as the miniature the soil particles, the

reduced passage of water and air through the soil), and acceptable biological environment for the seed germination in soil. Soil pH *(i.e.* acidity or alkalinity) also affects the success rate of seed germination. Soil that is hypertonic hinders the process of seed germination as high osmotic potential in the seed surrounding will put off water from progressing into the seed. Excessive fertilizers also inhibit seed germination.

6.4.5 Soil depth

Soil depth also has apparent effect on the success of seed germination as oxygen and light diminish with the depth of the soil. Minimal rate of germination at the soil cover can be recognized due to the greater water potential and longer imbibition time potential of the seeds for the process of germination.

6.5 SUMMARY

1. Ripened ovule is called as seed and the whole miniature plant exists in a seed.
2. Seed germination is defined as the emergence of embryo from the seed.
3. Seeds are the association between two consecutive generations of a plant.
4. In the life cycle of a plant, seeds have the highest resistance to acute environmental strains.
5. Plant hormones are physiological intercellular messengers.
6. Most important plant hormones for seed germination are Gibberellic acid and abscisic acid representing stimulatory and inhibitory effects respectively.
7. Cytokinins are effective at all phases of germination.
8. Moderate warmness is needed for the vital actions of protoplasm and consequently for seed germination.
9. Soil depth also has marked effects on the success rate of seed germination.
10. Reduction in percentage of seed germination by increasing salt stress is due to lethal consequences of sodium and chloride ions.
11. Water deficit/ excess water is also one of the key factors that can hinder the success rate of seed germination.
12. Gibberellic acid and ethylene increase the rate of seed germination by splitting testa and endosperm.

6.6 GLOSSARY

Growth: Irreversible increase in size, mass and volume.

Development: It is the process by which structure originates and establishes as plant grows.

Hormones: A substance that affects the course of growth and development.

Anoxia: Absence of oxygen.

Endosperm: A nutritive tissue found in the seed plants that provides nutrition to the growing embryo.

Quiescent: Phase of inactivity.

Secondary growth: Increase in the girth of a plant.

Phytochrome: It is a blue green pigment found in plant which controls several progressive activities.

Radicle: It is the portion of embryo that grows into a primary root.

6.7 SELF-ASSESSMENT QUESTIONS

6.7.1 Multiple choice questions

1. ABA is also termed as:

a. Stress hormone b. Growth enhancer

c. Both a and b d. None of the above

2. Which of the following hormone is referred as enhancer of seed germination?

a. ABA b. Auxin

c. GA d. None of the above.

3. Plant hormones are referred as

a. Growth hormones b. Physiological intercellular messenger

c. Both a and b d. None of the above

4. Ethylene is also referred as:

a. Gaseous hormone b. Solid hormone

c. Both a and b d. None of the above

5. Among which of the following represent the class of plant hormones similar to steroid hormones:

a. Brassinosteroid b. GA

c. Auxin d. ABA

6. Among which of the following hormone was isolated from the pollens of *Brasica napus.*

a. Auxin c. Ethylene

c. GA d. Brassinosteroid

7. The plant hormone "Cytokinin" was isolated from

a. Herring fish sperms DNA b. *G. fujikuroi*

c. Both a and b d. None of the above

8. Seeds are the characteristic feature of-

a. Angiosperms b. Gymnosperms

c. Both a and b d. None of the above

9. Among various auxins, which one of the following is able to influence seed germination?

a. IAA b. NAA

c. Both a and b d. None of the above

10. Which of the following hormone is responsible for fruit ripening?

a. Auxin b. ABA

c. Ethylene d. None of the above

11. Among which of the following hormone hinder the germination of seeds by slowing down the development of radicle and drooping of endosperm.

a. ABA b. Auxin

c. Both a and b d. None of the above

12. Which hormone is able to augment seed germination by the mitigation of stress.

a. Cytokinin b. ABA

c. Both a and b d. None of the above

13. Which set of hormones improves the capacity of embryo to emerge from the seed by splitting the endosperm and antagonistically relating with abscisic acid.

a. GA, Brassinosteroid and Ethylene
b. Auxin, Cytokinin and ABA
c. ABA, Auxin, Brassinosteroid
d. None of the above

14. Which hormone augments seed germination by impeding ABA activity triggered by the activity of catabolizing enzymes and hindering the related biosynthetic pathways which also lower abscisic acid quantity?
a. Gibberelic acid
b. ABA
c. Both a and b
d. None of the above.

15. Ripened ovule is referred as
a. Seed
b. Flower
c. Endosperm
d. None of the above

Answers key: 6.7.1: 1 (a); 2 (c); 3 (b); 4 (a); 5 (a); 6 (d); 7 (a); 8 (c); 9 (a); 10 (c); 11 (a); 12 (a); 13 (a); 14 (a); 15 (a).

6.7.2. Fill in the blanks:

1. Salinity stress leads to ……..in rate of germination.
2. GA stimulates synthesis of ……..
3. Germination under light condition may be due to the …….
4. ……. Seeded seeds are more sensitive to light for germination.
5…….can modify soil organization and its fertility.
6. ……. is one of the environmental factors that may control germination of seeds and initial formation of seedlings.
7. Seed germination is …….
8. …… also affect the success rate of seed germination.
9. Various environmental factor affecting the process of seed germination are…….
10. Stress hormone" obtained from ……

Answers key: 6.7.2: 1. Reduction, 2. Hydrolases especially α-amylases, 3. Active form of Phytochrome 4. Small, 5. Sodium ions, 6. Light, 7. Emergence of radicle from the seed coat, 8. Soil pH 9. Light, temperature, salinity stress, soil environment and soil depth, 10. Mature cotton fruits.

6.7.3. True and False:

1. ABA is called growth promoting hormone.
2. GA cause dwarfing effect.
3. Seed germination and seed dormancy are similar to each other.
4. Soil depth has marked effect on the process of germination.
5. Cytokinin is active at all stages of germination.

Answers key: 6.7.3: 1. False, 2.False, 3.False, 4.True, 5.True

6.7.4 Very short answer type questions

1. Define Plant growth hormones
2. Define seed dormancy.
3. Define seed germination.
4. Explain Phytochrome.
5. Define pH.

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6.10 TERMINAL QUESTIONS

6.10.1 Short answer type questions:

1. Describe the effect of auxins or cytokinins in seed germination.
2. Define salinity stress.
3. Define the role of brassinosteroids in plants.
4. How soil depth affects seed germination.
5. Explain role of ethylene in the process of seed germination.

6.10.2 Long answer type questions:

1. Describe in detail effect of different environmental factors in the process of seed germination.
2. Define role of different hormones in seed germination.
3. Define the biology of seed.
4. What is the difference between growth hormone and growth regulator? (Find in other books)

BLOCK-2 SEED BIOLOGY

UNIT-7: SEED STORAGE, DORMANCY AND GERMINATION

7.1 OBJECTIVES

After reading this unit students will be able to-

- Know about seeds and their storage
- Understand the concept of dormancy and methods to overcome it.
- Understand the different types of seed germination and factors affecting germination.

7.2 INTRODUCTION

In this chapter, we will discuss about the seed, its storage, dormancy and seed germination. Factors affecting seed storage, different methods to overcome seed dormancy and different types and factors contributing to seed germination. Seed in general explained as a fertilized ovule which develops inside the ovary (fruit). From morphological point of view, a seed is a mature integumented megasporangium. Seeds are enclosed within carpel in angiosperms but not in gymnosperms. Structurally seeds vary from plant to plant but the general structural organization always remains the same. Seed contains reserve food, enzymes, an embryonic axis bearing one or two cotyledons.

On the basis of embryo structure seeds are classified into

(i) seeds having an embryo with two cotyledons (Dicotyledonous) or
(ii) with one cotyledons (Monocotyledonous).

The embryonic axis consists of radicle which gives rise to root and plumule which gives rise to shoot. The reserve food in seed when present in the endosperm, the seeds are called as endospermic seeds (Maize, Wheat, Barley), while when present in cotyledons, the seeds are called as non-endospermic seeds (Pea, Bean Gram etc). The endosperm and cotyledons store large quantities of carbohydrates, lipids, proteins, growth promoting substances and other minerals. The seeds always remain surrounded by a layer called as seed coat, which develops from the integuments of the ovule. The outer layer of the coat is called as testa (composed of thick walled cells and is covered with thick waxy cuticle) and the inner layer of the seed coat is called as tegmen.

Seeds contribute major percentage of food for the human consumption, 70 per cent of the total food comes from the cereals and legumes for human beings. The collection of viable seeds for the future use is called as seed storage. Viability of seeds is the ability of the seed to retain the power of germination for some specific period of time. The viable seeds, when fail to germinate even under the germinating conditions are called as dormant seeds which may be due to a number of factors such as due to seed coat, condition of embryo, specific light conditions, temperature and germinating inhibitors etc. Seeds have also been considered as physiological enigma of the world and it is interesting to note that, the living cells die if water content is below 45-70 per cent, but seeds continue to live with 10-15 per cent water content.

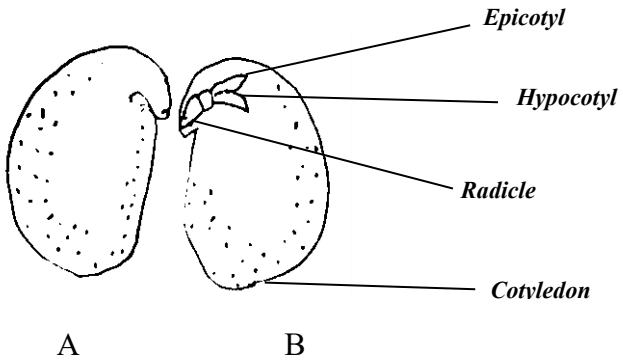


Fig 7.1: (A). External structure of seed; (B) seed coat removed

Seeds vary widely in their shape and size i.e., from the dust-like to barely visible seeds of orchids weighing between 0.3 and 14 µg to the largest of the *Lodoicea* which is almost 10 kg in its weight.

7.3 SEED STORAGE

Seed is a ripened ovule which contains the embryo and seed storage may be defined as the preservation of viable seeds from the time of collection until they are required for sowing. Collected seeds should be stored in such a manner that their vigour and germination capacity should not deteriorate during storage. Approximate 30% of the total seeds are lost due to insects, rodents and microorganisms during the course of seed storage. Thus seeds should be stored under good storage facilities so that seeds remain viable for longer time and damage should be minimum due to insects, pests etc, because seeds are the main genetic linkage between the two generations of the plant species.

7.3.1 Classification of Seed on the basis of their storage

On the basis of retaining the power of germination and viability, seeds were classified into number of classes. According to the time for which the seeds remain viable under "good" storage conditions, Ewart (1908) divided seeds into three biological classes i.e.,

1. Microbiotic: Seed with life span not more than 3 years.
2. Mesobiotic: Seed life span ranging from 3 to 15 years.
3. Macrobiotic: Life span of seeds ranging from 15 to over 100 years.

Although Ewart's classes were useful in drawing attention but his classification was too rigid to include the variations among the individuals and further, it was not possible to define a standard set of "good" storage conditions equally suitable to all species.

In another classification of seeds based on storage, seeds can be categorized into three types based on the longevity of the seeds during storage,

1. **Orthodox seeds**: Orthodox seeds or long lived seeds may be defined as the seeds which can be stored for a relatively longer period. The seeds can be dried up to 5% moisture level without any injury to their embryo and can also withstand freezing temperatures. e.g, Rice, Maize etc.
2. **Intermediate seeds**: Intermediate seeds can also be stored for longer periods, but they are not able to withstand low temperature. e g., Papaya, Citrus etc.
3. **Recalcitrant seeds**: Recalcitrant seeds or short lived seeds which can be stored only for a short span of time. Since they cannot be dried to less than 30% moisture level, chances of pest and disease incidence is more. It cannot withstand drying under direct sun. These seeds never get into dormancy stage, but continue to enter into the next stage of germination e.g. Mango, Jack fruit etc.

7.3.2 Care and preparations before storage

1. Seeds should be dried to optimum moisture level (4-14%); less than 12% for starchy seeds and less than 9% for oily seeds.
2. Seeds should be cleaned thoroughly and should be free from trash, insect and microbial damage.
3. Selection of storage containers should be reasonably inexpensive, airtight, moisture proof with low thermal conductivity.
4. The godown (storage place) should be clean and dry. Selection and maintenance of godown area should be easily accessible for loading and unloading operations and area should be termite and rodent proof.
5. Fumigation of storage godown should be done before storing seeds. Fumigation process should be done once a month during storage period.
6. The dead moths and insects should be cleared from the storage godown next day morning.

7.3.3 Factors affecting seed storage

Factors that affect stored grains can be classified into internal and external factors. Internal factors include kind and variety of seeds, seed quality and moisture content. The term external factor denotes temperature, light and activity of insects and other microorganisms in the storage area.

Seed condition

Even in ideal storage conditions, seeds may soon lose viability if it is defective from the start. Factors to be considered are:

a. **Seed maturity**: Seed maturity is regarded as the primary factor for seed storage. Fully ripened seeds contain certain important biochemical compounds, essential for preserving viability, which may not be formed until the final stages of seed ripening hence mature seeds retain viability longer than seeds collected when immature example *Ginko biloba*.

These important biochemical compounds include dormancy-inducing compounds and dormancy is sometimes associated with seed longevity.

b. **Seed damage:** Seed damage during extraction, collection, cleaning etc is also responsible for reducing viability of seeds during seed storage.

Storage conditions and ageing of seeds

Ageing is a universal phenomenon and explained as "Slow deterioration process with the age" and explains increased catabolism over anabolism in an organism or any living subject. Seeds are also subject to ageing and eventually, to death. In seeds, the process of ageing and deterioration is greatly affected by the conditions of their storage. Ageing in seeds is described as the degree of deterioration of seeds measured by their reduced capability for germination. As an example, Barley indicates that the degree of deterioration from initial 95 % to final 50 % in germination, occurred in about 16 days when seeds were stored at 25° C and 21 % moisture content, but when seeds were stored at 8° C and 8 % moisture content, the same happened in about 100 years in stored seeds.

Number of physiological changes that occurs in cell tissues may be associated with physiological ageing in seeds are:

(1) Loss of food reserves caused due to catabolism, e.g. decrease in proteins and non-reducing sugars accompanied by increase in reducing sugars and free fatty acids.

(2) Accumulation of some toxic or growth-inhibiting by-products of catabolism.

(3) Decreased activity of enzyme systems.

(4) Loss of ability of dried protein molecules to recombine to form active protoplasmic molecules on subsequent rehydration.

(5) Deterioration of semi-permeable cell membranes.

(6) Production of free radicals which react with other cellular component and damage them.

(7) Gene mutations (Roberts 1972, Harrington 1973, Villiers 1973).

Storage atmosphere

Suitable atmospheric conditions for the seed storage are those in which the rate of aerobic respiration is minimum and this can be achieved by replacing oxygen with the other gases such as nitrogen, argon etc, or storing seeds in vacuum. Robert (1972) recorded that, when seed of lettuce were stored for 3 years at 6 % moisture content and 18 ^{0}C temperature in pure oxygen they had 8 % viability, while those in air 57%, in nitrogen, argon and CO_2 78% and those in vacuum 77% viability. Other simple recommended method is to fill sealed containers as nearly full as possible. If there is a small amount of air inside the container as compared with the volume occupied by the seeds, oxygen will be consumed and

CO_2 produced. The resulting high CO_2/O_2 ratio is probably favourable for seed longevity in seeds storage.

Storage temperature and Moisture content

Temperature and moisture content are negatively correlated with seed longevity, thus low temperature and the lower rate of respiration there is the longer life-span of the seeds during storage. Optimum moisture content for seeds storage is 5-14%. Choice of storage temperature varies considerably according to species. Seeds of leguminous and some other can be stored at room temperature while in some low temperature such as 1 to 4 ^{0}C is required.

Light

Light is also one of the important factors to keep in mind during seed storage, particularly ultra-violet light which is harmful to stored seed. Thus use of opaque metal containers is recommended than the glass jars or other transparent materials. But light appears to be much less important than either moisture content or temperature.

7.3.4 Choice of storage methods

Storage Containers

For long term storage of seeds, some form of container is necessary, which facilitates easy access for handling of individual seeds and keeping them with the other seeds in the same godown so as to make the best possible use of storage space. Many different types of containers have been used for seeds storages and conveniently divided into- (1) Materials freely permeable to moisture and gases.
(2) Materials completely impermeable, when sealed to moisture and gases.
(3) Materials resistant but not completely impermeable to moisture.
Further, if during seed storage seed requires further drying, do not use a tight-closing container because enclosing excess moisture is harmful to the seeds. Use a tight-closing container if gain in seed moisture content can be damaging and relative humidity in the storage facility is high.

Materials freely permeable to moisture and gases

These containers include hessian or burlap (woven febric) sacks, cotton bags, containers of paper, cardboard and fiber board. None of these materials is entirely proof and freely permeable to moisture and gases. Hessian and cotton are robust materials which may used for more than once and have several advantages over others i.e., seed triers can be inserted through the cloth mesh to withdraw samples for testing without opening the mouth of the container. The resilience of the cloth will close the hole and avoid subsequent loss of seed which is not possible with containers based on paper or paperboard. These types of container are not the best to store orthodox seeds but fits best for the storage of recalcitrant

seeds because these allows free exchange of air. Rapidly respiring seeds are enclosed in containers that are without adequate ventilation.

Materials completely impermeable when sealed, to moisture and gases

These types of containers are very effective for the orthodox seed storage but are not suitable for the recalcitrant seeds. Moisture proof containers include tin or aluminium cans and drums, glass jars of the Mason or Kilner types, plastic vials and laminated aluminium foil packages. For long-term storage of the seeds this system is very effective method is a combination of moisture proof containers with controlled low temperatures provided by refrigeration.

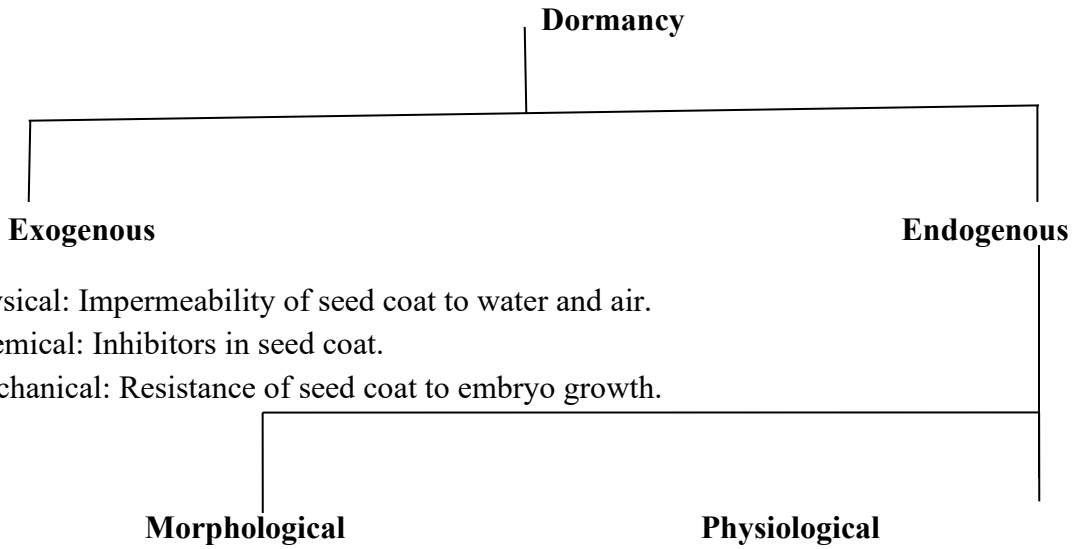
Materials resistant but not completely impermeable to moisture

These include polyethylene and other plastic films and aluminium foils. These materials are resistant to the passage of moisture but, over a long period of time, there will be a slow passage of water vapours tending to equilibrate the RH inside with that of outside the container. Although polyethylene is not suitable for long-term storage of orthodox seeds for genetic conservation. It is very suitable for short-or medium-term storage and has given excellent results.

7.4 SEED DORMANCY

Dormancy is a phase in the life cycle of plants in which their active growth is temporarily suspended to overcome the unfavourable conditions (winter frost, summer drought) so that the chances of their survival may enhance. In different plants dormancy is represented by different conditions such as resting spores in lower plants and seed dormancy, bud dormancy in higher plants.

Seed dormancy means the failure of seeds to germinate although environmental conditions including water, temperature, light and gases are favourable or the inability of a viable seed to germinate under favourable conditions. In nature there are two general kinds of seed dormancy i.e., endogenous and exogenous (Nikolaeva, 1969, 1977). In endogenous dormancy some characteristics of the embryo prevents its germination, while in exogenous dormancy some growth inhibitors or structural features such as seed coat or fruit wall, or covering of the embryo prevents their germination.



7.4.1 Factor causing seed dormancy

The factors contributing to the seed dormancy can be explained under the following headings:

1. Due to seed coat: Seed coat commonly called as testa, is tough protective layer around the embryo and endosperm. The mature seed coat is differentiated into five layers i.e., l_1 (epidermis), l_2 (hypodermis), l_3 (mechanical layer), l_4 (aerenchyma), l_5 (chlorenchyma). This protective layer of seed coat is made of a complex mixture of polysaccharides, wax, fats and proteins. Primarily seed coat's function is to protect the embryo and endosperm from the mechanical damage, injury, microbial attack etc, and also help in seed dispersal as the seed coat of some seeds are expanded into wings *(e.g., Bignona)*, and some times may provide nutrition to the developing embryo (*Pisum*). Besides this, several times it acts as barrier for the seed germination.

In some cases, during the seed ripening, chemical component of seeds coat become dehydrated and form a hard, tough protective layer around embryo. This several layered seed coat serves as a barrier for the seed germination (legumes). In several other plants seed coats are impermeable to water (*Melilotus*, *Trigonella*), since water is the primary factor for germination, seeds fail to germinate. Impermeability to water is also wide spread in the legumes. Further, as the seeds become drier the permeability of the seed coat to water decreases and the dormancy of the seeds increases. Besides this, in some others, seed coat is impermeable to gases such as oxygen and carbon dioxide. Differential permeability for gases (oxygen and carbon dioxide) is well known and established in cocklebur (*Xanthium*) by Shull. Shull established that *Xanthium* seeds are more permeable to carbon dioxide then the oxygen and permeability may be increased by saturating the seed coat with water, in this plant fruit contains two seeds an upper dormant seed and a lower non- dormant seed and seed coat in this case act as barrier for non uptake of oxygen. Further, in some plants,

the seed coat is hard and tough that cannot be broken by the germinating embryo or contain some germinating inhibitor which results in seed dormancy.

2. Due to condition of embryo: Embryo condition is the primary factor for seed dormancy. In the seeds of several plants, the embryo in seeds are present in rudimentary condition which requires a period of rest to form fully mature embryo, if such seeds with rudimentary embryos were sown they fail to germinate (Orchidaceae, Rosaceae and in few members of Ranunculus). Besides this, in some cases seeds with fully mature embryo require resting period before germination i.e. a period of after ripening, which improves the germination percentage (*Malus, Rumex, Fxaxinus*), if freshly harvested seed were sown they show no germination or poor germination.

3. Due to light requirement: the effect of light on the seed germination is very diverse. The seeds respond to sunlight can be grouped under three categories: **Positive photoblastic** seeds (germinates on exposure to light), **negative photoblastic** (seeds fail to germinate when exposed to light), **neutral** or **non photoblastic** (germinates in dark as well as light). Further, in lettuce seeds germination is stimulated by red light, in *Begonia* seed germination requires photoperiod of 12 hours.

4. Temperature and chilling requirement: Temperature also acts as detrimental factor for the seed germination. In some plants seeds do not grow when placed under constant temperature but germinates when temperature is fluctuating, example *Ricinus obtusifolia, Rumex*. Besides, alternating temperatures, in case of seeds of many temperate plants a chilling temperature is required before they become capable of germination, this chilling temperature satisfies the germination requirement, example *Acer, Pyrus, Malus*. The most suitable temperature for chilling treatment is 0-5^{0}C. In natural conditions chilling treatment to seed can be received in winter.

5. Germination inhibitors: In certain seed plants dormancy is due to presence of number of growth inhibitors such as abscisic acid (ABA), phenolic acid, parascorbic acid, ferulic acid and coumarin etc which inhibit the plant growth in general and seed germination in particular. Besides this, some germination inhibitors are leached in the soil by the plants (generally weeds) for example *Lantana sps* which inhibit the germination of the seeds of other plants. This kind of inhibition is also called as **allelopathic inhibiton**.

7.4.2 Secondary dormancy

Under the germinating conditions, seeds germinate immediately after they shed. But in some cases it was reported that seeds lost their germinating ability spontaneously due to unfavourable conditions. This is known as secondary dormancy which can be overcome by providing favourable conditions.

7.4.3 Methods of breaking seed dormancy

The dormancy of the seeds can be broken by number of methods enabling the dormant seeds to germinate as a normal seed.

1. Scarification: The dormancy of seeds due to its hard seed coat can be removed by removing the seed coat, or by softening the seeds and by the action of microbes, or certain chemicals and by cutting or rupturing the seed coat. Under natural conditions, the seed coat is usually weakened by the action of soil microbes. Scratching, cutting or chipping the seed coat, using certain chemicals and acid treatment are highly recommended in *Sapindus*.

2. Impaction: In plants like *Crotalaria, Melilotus* etc. seed coat is impermeable to water and oxygen due to blockage of the opening of seed coat. In such cases, the seeds are shaken vigorously to remove the plug which facilitates the passage of water and oxygen. This treatment to overcome dormancy is called impaction.

3. Stratification: Some seeds (cheery, apple etc) require the treatment of moist condition under low temperature (0^0C-10^0C) from a week to month before germination. This treatment is required to overcome the rudimentary embryo condition of seed. It is also reported that during the stratification, the level of ABA decreases from the normal seeds (*Fxainus, Juglans*). In *Acer* sp increase in the gibberellic acid coupled with the decrease in ABA content is reported in those seeds which receive stratification treatment.

4. Alternating temperature: The germination of certain seeds (due to condition of immature embryo) is promoted by daily temperature variations.

5. Light

The dormancy of positive photoblastic seeds can be broken by exposing them to red light (660 nm). Far-red light inhibits the seed germination indicating the involvement of photoreversible pigment phytochrome in the process of seed germination. This pigment occurs in two forms, one red absorbing and other far-red absorbing. Both these forms are photochemically interconvertible. The red absorbing form (P_R) is converted into far-red form (P_{FR}) after absorbing the red light. The far-red form absorbs the far-red light and is converted back into red absorbing form of the pigment.

lt is supposed that in positive photoblastic seeds, the far-red absorbing form of the pigment is stimulatory to seed germination while red-absorbing form is inhibitory to seed germination.

6. Pressure:

The seed germination in certain plants like sweet clover (*Melilotus alba*) and alfalfa (*Medicago sativa*) can be greatly improved after being subjected to hydraulic pressure of about 2000 atm. at 18°C for about 5-20 minutes. This pressure changes the permeability of seed coat to water resulting into seed germination.

7. Growth Regulators:

Growth regulators are most widely used to hasten the development of roots or cuttings and to increase the number of roots. Kinetin and gibberellins have been used to induce germination in positively photoblastic seeds like lettuce and tobacco etc. Besides, a number of chemicals such as KNO_3, thiourea and ethylene etc. have also the capacity to induce seed germination.

8. Other: In some seeds, the dormancy can be removed by placing the seeds under running tap water, treating the hydrated seeds with oxygen, nitrite etc.

Advantages of Seed Dormancy

1. The dormancy of seeds helps the plants of temperate zones to tide over the severe colds.
2. The dormancy of seeds due to impermeable seed coats ensures good chances of survival to the plants of tropical regions.
3. The dormancy of seeds in cereals is most important to mankind. If these seeds germinate immediately after harvest, they will be quite useless for mankind.
4. Dormant seeds and organs in perennial plants resist unfavourable conditions for their development.
5. The seeds form a measure of the quantity and duration of rainfall, both of which determine the amount of soil-moisture available for plant growth.

7.5 SEED GERMINATION

Seeds are specialized structures having an enclosed embryo within its seed coat. Seeds are structurally and physiologically equipped for dispersal and are well provided with food reserves to sustain the growing seedling until it establishes as an independent photosynthetic unit. In Botany **"Emergence of radicle from seed coat"** is referred as seed germination. A new plant generation starts with the formation of seed. Kernel (embryo) is the future plant in miniature condition. By sowing a mature, viable seed in soil, a new plant arises from the embryo. Thus, when supplied with all necessary conditions, the seed spout into a new plant. During the course of seed germination physiological activities and enzymatic activity within the seed increases and stored food in the storage organs of the seeds are converted into soluble forms and transported to the sites of requirement where they are utilized by the developing embryo until it becomes photosynthetically efficient. The embryo in the seed is made up of embyonal axis, which contains radicle (embryonic root) usually growing first and plumule (embryonic shoot). Thus, seed germination may be defined as the process by which dormant embryo wakes up, grow out of the seed coat and establishes itself as a seedling. Further, based on the behaviour of cotyledons there are two types of germinations i.e., **epigeal** (due to rapid growth, the hypocotyl elongates carrying the cotyledons and the plumule out of the soil) and **hypogeal** (in this case hypocotyl does not elongate, the epicotyl pushes plumule upward while the cotyledon remains in the soil).

The seed is the structure in which usually a fully developed plant embryo is present and which enables the embryo to survive the period between seed maturation and seedling establishment, thereby ensuring the initiation of the next generation. The dry dormant seed is well equipped to survive extended period of unfavourable conditions. Seed dormancy is defined as the failure of an intact viable seed to complete germination under favourable conditions and is controlled by several environmental factors, such as light, temperature and the duration of seed storage (after ripening). Dormancy and germination both are determined by the co-action of the growth potential of the embryo and the restraints imposed by the tissues surrounding it.

7.5.1 Types of seed germination

1. **Epicotylic germination:** In this type of germination the hypocotyl is well developed and it exposes the cotyledons to light as in *Costus gpeetosus*. With the start of enzymatic digestion of stored food, the soluble food starts translocating to hypocotyl, plumule and the radicle. As a rule, radicle emerges first, which is followed by the rapid growth of hypocotyl. Elongation of hypocotyl in all cases results in the formation of hypocotylar loop which straightens up and exposes the seed coat and the cotyledons to light and carries two cotyledons and the plumule out of the soil. During this course, the plumule develops fast and forms foliage leaves. Radicle shows positive geotropic growth and bends downward to form root, example seeds of castor, cotton, bean etc.

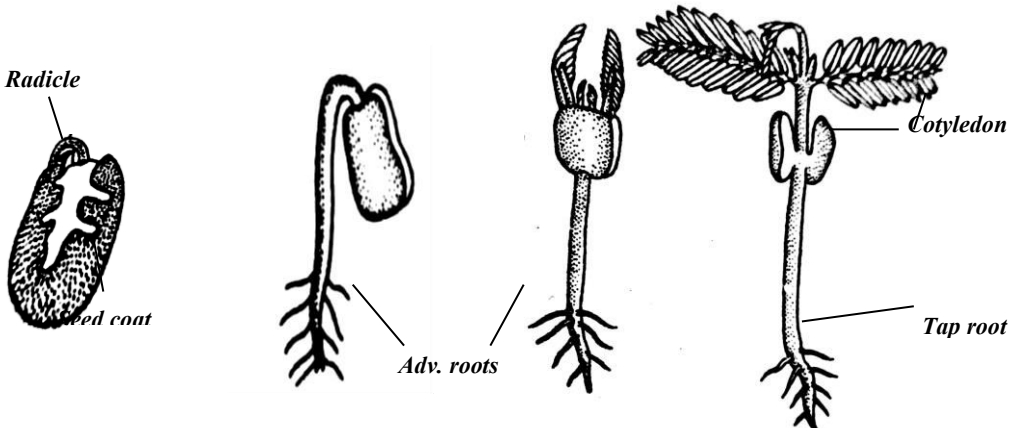


Fig7.2: Stages of epicotylic germination in Tamarind seed

2. **Philomenean**

It can equally be called as semihypogeal or semiepigeal type of germination. In this process of germination, the seeds showe the mixture of both epicotylic and hypocotylic characters. During the course of germination, cotyledons are exposed as in case of epigeal germination and hypocotyl is absent or poorly developed as in hypogeal germination (*Allium cepa*).

3. **Hypocotylic germination**: In this type of germination the hypocotyl is undeveloped and the cotyledonary regions are hidden beneath the ground. Unlike the former types the seedling remains parasitic on the food reserves in the seed, thus heterotrophic for a long time e.g. *Aloe barbadensis* and *Asparagus officinalis*.

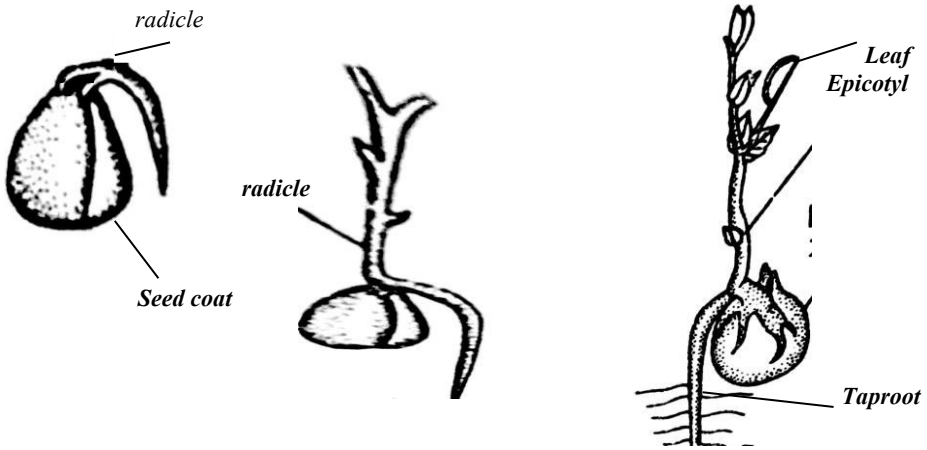


Fig 7.3: Stages of hypocotylic germination in pea seed

4. **Cryptogeal**

In this type of germination the hypocotyl is undeveloped and the cotyledonary region is hidden in the seed beneath the ground and the embryo axis is pushed into the ground by the elongating apocole (cotyledonary middle piece along with the sheath). As a result the shoot arises from below the ground even if the seed is germinated on the surface e.g. *Phoenix dactylifera*

5. Durian

In this condition, when the hypocotyl is developed and the cotyledonary region is hidden completely in the ground or partially exposed. The hypocotyl lifts the plumule to the ground level and during lifting seed body is avoided by the development of long cotyledonary stalks connecting the seed body in the ground to the elevated cotyledonary node as in *Tradescantia discolor*.

6. Mesogeal

In this condition the hypocotyl does not develop but is substituted by another specialized axis known as the mesocotyl which lifts up the plumule to the ground level.

7. Anomalous type

Anomalous type of seed germination is also reported in several spp, in which besides the normal germination i.e., radical emerges out of the seed first as a sign of germination, emergence of the plumule or coleoptiles is seen prior to the radical in taxa like *Chlorls gayana*, *Majas marina* and *Nytndftaea lotus*.

9. Viviparous

Vivipary is the phenomenon of germination of seeds while still with the plant or in other words, vivipary in flowering plants is defined as the precocious and continuous growth of

the offspring when still attached to the maternal parent. Vivipary has been reported and described in 23 families and 40 genera of the plants. It is commonly reported in *Rhizophora, Ceriops, Polygonum viviparum, Agave* spp*, Crinum viviparum* etc. In all these plants seed germinates *in-situ*. In some plants like tomato, melon, orange etc. the vivipary is accidental.Vivipary is found in halophytes (Mangrove plants).

7.5.2- Factors regulating seed germination

1. Water: Mature seeds usually contain 05-20 per cent of water except in some larger seeds of mapal, acrons, citrus as they may contain water content upto 50% which help the seeds to remain in dormant condition. But for the germination of seeds, the seed protoplasm must be saturated with water. Seeds absorb water by imbibition resulting the swelling of seeds and breaking of seed coat. Water dissolves many salts and hydrolyses many organic substances stored in the cotyledons. Further, water rehydrates number of cellular constituents and facilitates necessary reactions in the embryo.

2. **Oxygen**: Imbibition of water is responsible for increase O_2 uptake in the seeds of *Xanthium* and *Cucurbita pepo* (Shull 1914; Brown 1940) and resumption of metabolic activity in dormant seeds. In the germinating seeds when embryo resumes its growth huge amount of energy is required, hence respiration takes place and when O_2 becomes a limiting factor, proper growth does not take place.

3. **Favourable temperature:** Temperature requirement varies from plant species to species, but the standardize temperature for maximum plants revolve around 25-30 ^{0}C. During seed germination metabolic events are at the peak, which require an optimum temperature for the effective working of enzymes within the seeds and it has been reported that the rate of germination increases steadily with increasing temperature up to an optimum which in most cases lies between 15° and 30°C. Fluctuating temperature is also known to enhance the speed of germination in some seeds as described earlier.

7.6 SUMMARY

In this unit we have discussed about the seed structure, its types, its storage, dormancy and germination. Seed is ripen ovule which forms the genetic link between present and future generation. Besides this, seeds form the major part of food for the world. Dormancy in seeds is of primary importance. It is for ensuring seed germination only under favourable conditions and to ensure the agricultural security which enables seeds to be disseminated in time and space. Dormancy is a phase in the life cycle of plants in which their active growth is temporarily suspended to overcome the unfavorable conditions. It is also for ensuring the world food security. To achieve the food security, seeds are generally stored for their long run use in future under the good storage faculties. Because during storage major losses encounters in the form of loss in viability of seed, insect, pest and other pathogens attack during the seed storage. On the basis of seed storage seeds are classified into three different types i.e., orthodox, intermediate and recalcitrant. Seed storage provides further the genetic

conservation of species. Seed dormancy, the term means the failure of seeds to germinate although environment conditions are favourable for germination. Seed dormancy is generally caused by seed coat (seeds are impermeabile to water or gases), condition of embryo (embryo are immature, or after-riping period is required), due to temperature, germinating inhibitors in the seed coat or sometimes in the embryo itself and due to light. Light is not a primary factor of the seed germination, but in rare cases the seed germination is under the influence of light and the seeds are called as photoblastic seeds. Dormancy can be overcome by different methods such as scarification, impaction, stratification, alternating temperature etc. and all these methods help in removing seed dormancy allow the seeds to germinate. Seed germination literally means the process by which the dormant embryo wakes up, grows out of the seed coat and establishes itself as a seedling. During the course of seed germination seeds undergo different germination pathways but most commonly follows the three i., hypogeal, epigeal and viviparous.

7.7 GLOSSARY

Dormancy: The failure of a viable seed to germinate under favourable conditions.
Durian: When the hypocotyl is developed and the cotyledonary regions are hidden completely in the ground or partially exposed.
Endosperm: The endosperm is the nutritive tissue present inside the seed.
Germination: The emergence of radical from seed after sowing it is called germination.
Scarification: The method of softening or weakening the seed coat is known as scarification.
Seed: Seed is a ripened ovule which contains the embryo or the miniature of future plant body.
Seed storage: The preservation of viable seeds from the time of collection until they are required for sowing.
Viability: The ability of the seeds to retain the power of germination.

7.8 SELF ASSESSMENT QUESTIONS

7.8.1 Multiple choice questions:

1. The dormancy in the seeds of orchids is due to
a. Hard seed coat b. Immature embryo
c. Germination inhibitors d. None of the above

2. Seed dormancy is due to
a. ABA b. Ethylene

c. Starch d. Gibberellic acid

3. The seeds of *Xanthium* are almost impermeable to
a. Water b. Both of these
c. Gases d. None of these

4. Some seeds or plant parts leach out chemicals which inhibit the germination of seeds of the other plants. This phenomenon is called
a. Competitive inhibiton b. Competitive amansalism
c. Allelopathy d. Competition

5. Viviparous germination is reported in
a. *Rhizophora* b. *Sonneratia*
c. *Avicennia* d. all of the above

6. The embryo in sunflower has
a. No cotyledon b. One cotyledon
c. Two cotyledons d. None of the above

7. Germination in the seed is promoted by
a. Green light c. Infra red light
b. Blue light d. Red light

8. During germination the cotyledons remain underground in
a. Castor seed b. Broad bean
c. Pea seed d. Dwarf bean

9. The seeds which can be stored for longer period is called
a. Orthodox seeds b. Intermediate seeds
c. Recalcitrant seeds d. None of the above

10. Which of the following is not germination inhibitor?
a. Auxin b. Abscisic acid
c. Coumarin d. Ferulic acid

7.8.2-Answer key:

1. (a); 2.(a); 3. (c); 4. (c); 5.(d) 6. (c) 7. (d) 8.(c); 9. (a); 10. (a).

7.8.2. Short answer type questions.

Q1. What is seed dormancy?
Q.2. What do you mean by seed germination?
Q.3. What are different types of seed germination..

Q.4. What you mean by seed viability?
Q.5. What is vivipary?
Q.6. What are orthodox seeds?

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7.10 SUGGESTED READINGS

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7.11 TERMINAL QUESTIONS

Q.1. Define seed. What are the various types of seeds on the basis of seed storage?
Q.2. Discuss in brief the procedure of seed storage and factors affecting seed storage.
Q.3. Give a brief account on dormancy in seeds and various methods employed to overcome it.
Q.4. What is seed germination? Describe its various types and factors of their regulations.
Q.5. Write in brief about the practical applications of seed dormancy.

BLOCK - 3

EXPERIMENTAL EMBRYOLOGY

Unit-8: APOMIXIS, POLYEMBRYONY AND PARTHENOCARPY

8.1 OBJECTIVES

After reading this unit students will be able to-

- Understand the concept of apomixis and its advantages.
- Understand the origin and types of apomixes.
- Understand the concepts of polyembryony and different modes of its origin.
- Understand the concept and types of parthenocarpy.

8.2 INTRODUCTION

Plants serve pivot role in grooming and unfolding human civilization since aeon and looked as a source of all goods to serve human race. In this unit we are going to discuss about some exceptions of plant embryology i.e. Polyembryony, Apomixis, and Parthenocarpy.

Reproduction is one of the most important functions of all living organisms. Sexual reproduction involves the formation of haploid (*n*) gametes through meiosis and the production of a *2n* zygote by syngamy. Meiosis primarily is responsible for the segregation and recombination of genes which results in new genome recombinations. Sexual reproduction probably evolved shortly after the origin of the eukaryotes from asexual prokaryotic ancestors. Understanding plant reproduction is of fundamental practical importance for improving the utility of the plants.

Angiosperms (flowering plants) form the largest division in the plant kingdom comprising about 270,000 species distributed almost on the entire globe, where ever life is present. They are incredibly diverse in their shape and size ranging from species of *Eucalyptus* trees well over 100 meters tall to some duckweeds that are barely millimeter in diameter. Other forms include epiphytic vines, succulent cacti, non-photosynthetic parasites and insectivorous plants. These forms are also major food source for animals and humans, as well as major economic source for the production of various commercial products. In angiospermic plant, flowers are the reproductive structures and concerned with the production of reproductive cells such as eggs and pollens which produce seeds containing the embryo and link one generation with the other (next). Seeds also contain endosperm formed as a result of triple fusion.

During the course of germination embryo gives rise to young plant called seedling where as the endosperm provides nourishment to the developing embryo. In general, after fertilization only one embryo is formed in the embryo sac but some time more than one embryo is present and the condition is known as **polyembryony** (first time reported by Leuwenhoek). Besides this, sometimes fruits develop without fertilization and this

condition is called **parthenocarpy**. Further, when seeds and embryo are formed without fertilization this process is called **apomixis**.

8.3 APOMIXIS

In natural sexual reproduction, sexual cycle involves two events i.e., Meiosis and Syngamy. Meiosis transforms the diploid cells to haploid gametes, which by syngamy gives rise to diploid zygote and the zygote after embryogenesis produces an embryo. But in some cases this natural sexual reproduction is interrupted and even then a viable embryo is formed. Such seeds which are formed without fertilization are called apomitic seeds and the phenomenon is called as apomixis. In botany, the term apomixis was defined by Winkler (1908) as replacement of the normal sexual reproduction by asexual reproduction (without fertilization or asexual seed formation). As this definition does not mention meiosis. Hence "normal asexual reproduction" of plants has never been considered to be apomixis. The term apomixis is commonly used as a synonym of Agamospermy (Richards 1997). Brown (1978) defined apomixis as production of asexual seeds in the absence of sexual fusion between gametes or 'seeds without sex'. Richards (1986) explained apomixis under following components: (i) the absence of meiosis, (ii) embryogenesis without fertilization (parthenogenesis) and (iii) functional endosperm development with or without triple fusion. Ostenfeld and Raunkiaer (1903) reported certain male sterile biotypes of *Taraxacum* and *Hieracium* which set ample seeds even when no pollen plants were growing nearby, if present they are far and wide. The plants derived from such seeds were genetically identical to their respective mother plants suggesting their apomictic nature. In nature, apomixis is widely spread in the flowering plants and occurs in 40 angiosperm families and 400- 450 species (Nogler 1984). Besides this, occurrence is most common in the members of families of Asteraceae, Poaceae, Rosaceae and Rutaceae which is 75% of total reported cases in angiosperms.

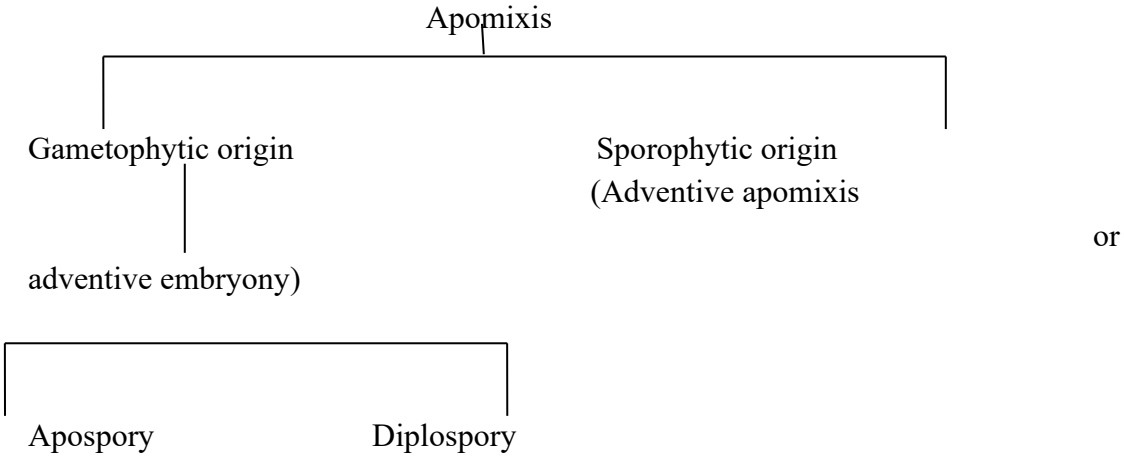
8.3.1 Mechanisms of apomixis

Ernst has pointed out that hybridization is the primary cause of chromosome aberrations. During the course of gametophytic apomixis three major developmental components are observed-(i) generation of a cell that are capable of forming an embryo sac without the meiosis, (ii) fertilization-independent development of the embryo and autonomous development of the endosperm or an endosperm resulted after the fusion of male gamete with polar nuclei. In sporophytic apomixis, embryos arise spontaneously from cells of the ovule late in the sequence of ovule maturation. Hence, the two methods differ mainly in the development and the type of cell involved in the development of an embryo.

8.3.2 Types of Apomixis

There are different types of apomixis as explained by different workers :

On the basis of developmental pattern of embryo and endosperm, two different types of Apomixis (agamospermy) are recognized.



Gametophytic origin: In gametophytic apomixis, the embryo sacs are produced from unreduced cells. Egg develops parthenogenetically. On the basis of origin of megagametophyte two major apomixis are apospory and diplospory. In first, unreduced (2n) female gametophyte are formed mitotically from nucellar cells, while in diplospory megasporocytes (megaspore mother cells) develop into mature unreduced female gametophyte. In either case embryo sac is diploid with all the cells inside as diploid.

Adventive apomixis: Adventive embryony commonly occurs in *Citrus* spp., Mango (*Mangifera indica*), *Opuntia dillenii, Trillium undulatum*, and *Commiphora wightii*. Further, different types of adventive embryony were described by Naumova (1981) in relation to the form of the ovule *viz.,* nucellar embryony reported to occur in crassinucellate ovules, e.g., *Citrus, Opuntia,* etc., while integumentary polyembryony is reported in tenuinucellate ovules e.g., *Euonymus* sp).

On the basis of presence or absence of sexual reproduction in apomictic species there are two different types of apomixis:

1. **Obligate apomixis**: if sexual reproduction occurs along with apomixis.
2. **Facultative apomixis**: when sexual reproduction is absent and only apomixis occurs, examples, C*enchrus ciliaris* , *Citrus* cultivar

Maheshwari (1950) recognized following three types of apomixis

(1) Adventive apomixis (2) Non-recurrent apomixis (3) recurrent apomixis

1. Adventive Apomixis or Adventive Embryony

Adventive embryony, also sometime called as sporophytic budding is a type of agamospermy in which the alternation of haploid and diploid generations is completely eliminated. The embryo develops from any cell of the ovule lying outside the embryo sac. *Citrus* is the most common example of the adventive embryony. Besides citrus, the adventive embryo is of common occurrence in Buxaceae, Euphorbiaceae, Cactaceae, Orchidaceae and Myrtaceae. The adventive embryony usually leads to the formation of more than one embryo in the seed and may grow simultaneously in the same embryo sac. The adventive embryo lacks any suspensor, which is present in the zygotic (normal) embryo. During the course of development, normal embryo competes with the adventive embryo or may degenerate.

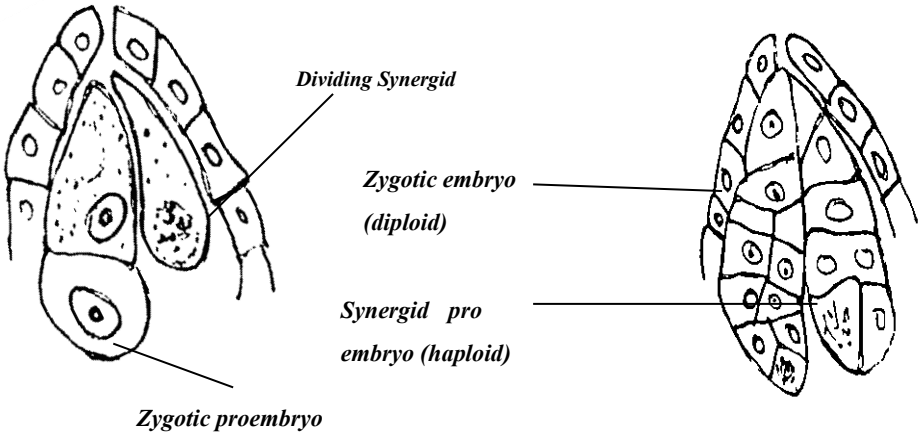
Sporophytic cells of nucellus or integument forming adventive embryo become densely cytoplasmic and divide actively to form a small mass of growing cells which pushes themselves into the normal embryo sac and undergo the stages of the embryogeny and develops into a mature embryo inside normally developed embryo sac getting nourishment from the endosperm present in the embryo sac.

2. Non -recurrent Apomixis

Non-recurrent type of apomixis is of rare occurrence and in this an embryo arises directly from normal egg-cell (n) without fertilization (haploid parthenogenesis) or from some other cell (synergid or antipodal) of the embryo sac (haploid apogamy). Since an egg cell is haploid, the resulting embryo will also be haploid. Haploid parthenogenesis and haploid apogamy and androgamy fall in this category.

a. Haploid parthenogenesis: In this type of apomixis, the developing embryo originates from the unfertilized egg. Jorgensen (1929) has shown that by stimulating the egg but not fertilising it with male nuclei, the haploid parthenogenesis can be achieved in some species of *Solanum*.

b. Haploid apogamy: In this type of apomixis, the development of embryo takes place from the cells of the embryo sac apart from the egg cell. This type of apomixis is reported from *Bergenia delavayi, Lilium, Erythraea* etc, in these cases twin embryo is observed and of the two one develops by normal fertilization and second develops from haploid synergid cell (fig 8.10).



Fig, 8.1: A & B, Haploid apogamy in Lilium.

3. Recurrent Apomixis

In this case extra embryo sac may develop directly from the megaspore mother cell or archesporial cell without undergoing meiosis. Thus the whole embryo sac is diploid and consequently, the egg-cell is diploid. The embryo develops directly from the diploid egg-cell without fertilization. Somatic apospory, diploid parthenogenesis and diploid apogamy are recurrent apomixis. However, diploid parthenogenesis / apogamy occur only in aposporic (somatic) embryo sacs. Therefore, it is the somatic or diploid apospory that constitutes the recurrent apomixis. Such apomixis occurs in some species of *Crepis, Taraxacum, Poa* (blue grass), and *Allium* without the stimulus of pollination. While in *Malus* and *Rudbeckia* pollination appears to be necessary and role of pollens is either to stimulate embryo development or to produce a viable endosperm.

8.3.3 Applications of Apomixis

In sexual reproduction meiosis reduces the chromosome number of the MMC to form four haploid megaspores and as a rule one of which develops into an embryo sac, but apomixis does not involve meiosis and there is no segregation and recombination of the chromosomes thus once regarded as evolutionary dead. Besides, apomixis is very useful in:

(i) Fixation of heterosis in crops or any genetic combination.
(ii) Fixation of pure heterozygous line as there is no meiosis and recombination.
(iii) Production of genetically identical plants to the female plant.
(iv) Used in the production of uniform root stock in *Citrus.*
(v) Production of phenotypically stable population of hybrids (progeny of two apomictics).

8.4 POLYEMBRYONY

Polyembryony may be defined as the occurrence of more than one embryo in the seed. This phenomenon was first time reported by Leeuwenhoek (1719) when he found orange seeds each containing two embryos. But it was Strasburger (1878) who first demonstrated the nature of polyembryony in *Citrus, Coelebogyne, Funkia*, and *Nothoscordum*. Since then, polyembryony has been reported in many taxa. Except for few taxa (*Mangifera*, *Citrus*) polyembryony occurs as an abnormal mode of embryo formation. In nature, the frequency of polyembryony is far more than the percentage of polyembryony in mature seeds. Additional embryo not always mature, there growth may check in the early stages or may degenerates during the course of the seed development and maturation. The polyembryony may be spontaneous or induced. Ernst distinguishes two classes of polyembryony: true and false. The production of plural embryos within, or by projection into the single embryo sac is designated as true polyembryony. While false polyembryony is the production of plural embryos derived from several embryo sacs. The hybridization is the primary cause of chromosome aberrations and responsible for polyembryony in triploid apple was recently attributed by Dermen. According to some, polyembryony is zygotic origin. In *Linum*, Kappert concluded that polyembryony is a recessive character probably conditioned by a series of multiple factors which are merely brought together in suitable recombinations following hybridization.

Thus, polyembryony should include only the accessory embryos arising from the zygotic proembryo.

8.4.1- Classification of polyembryony

1. Ployembryony is classified into various ways by the different authors but broadly polyembryony is of two types:

Polyembryony

Spontaneous
(Naturally occurring polyembryony)

Induced
(Experimentally induced)

Ernst (1901) sub-divided spontaneous polyembryony into two:

Spontaneous polyembryony

True polyembryony
(additional embryo arises in the same embryo sac)

False polyembryony
(more than one embryo in the same ovule)

2. Yakovlev (1967) proposed another classification of polyembryony on genetic basis:

Polyembryony

Gametophytic
(Arising from any gametic cell) the cell of the ovule

Sporophytic
(From the Zygote, proembryo or sporophytic cells of the ovule)

8.4.2 Types of polyembryony

Barun (1859) after survey of 60 cases of polyembryony from the literature at that time referred them to four different types on the basis of origin of the additional embryo in the seeds of the angiosperm.

(1) Cleavage polyembryony.
(2) Formation of the additional embryo by cells of the embryo sac other than the egg cell.
(3) Development of more than one embryo sac within the same ovule.
(4) Development from the activated cells of sporophyte of the ovule.

1. Cleavage Polyembryony

Cleavage polyembryony is accomplished by the splitting of the zygote or young embryo into two or more units, each of which develops into a separate embryo.Cleavage polyembryony develops due to the splitting of the proembryo, which was first reported by the Jeffery (1895) in the *Erythronium americanum* a member of Liliaceae. In this, cleavage of the zygote or its derivatives leads to the formation of the separate embryonal primodia. Cleavage polyembryony is of widespread occurrence in the gymnosperms, but less frequently reported in angiosperms. But among reported angiosperms it is quite common in orchids. Since the embryos in cleavage polyembryony are monozygotic in origin, the resulting seedlings are identical.This view is supported in *Linum* (Linaceae) by multiple seedlings that were genetically identical as evidenced by both flower colour and multiple characters. Cleavage polyembryony may arise in other ways also. In *Exocarpus* and *Isotoma,* the developing polyembryos develops from the suspensor cells of the proembryo

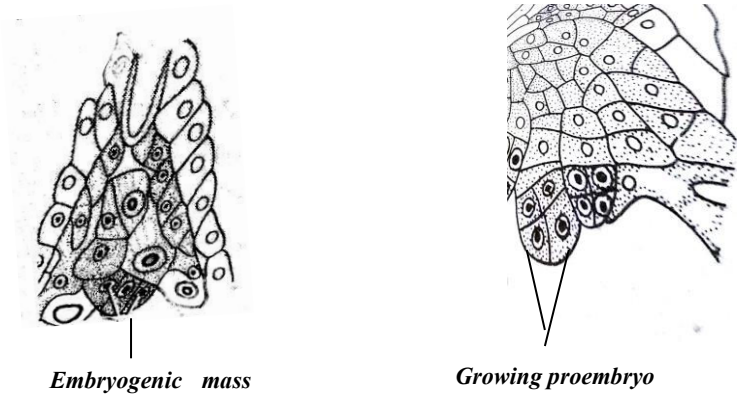


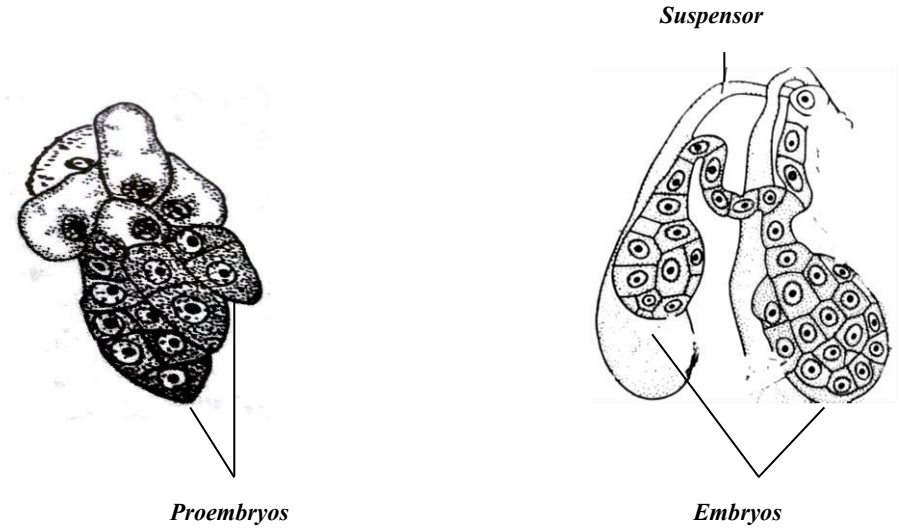
Fig 8.2: cleavage polyembryony in Erythronium americanum: (a). basal cell of zygote producing embryonic mass; (b). proembryo formation from embryonic mass.

In *Erythronium americanum* the additional embryos develop form the embryonic mass in which some cells at the distal end separate and contribute in the formation of embryo which is formed as the result of repeated division in the basal cell of the zygote (fig 8.2). *Tulipa gesneriana, Vincetoxicum* spp*.,* are some other examples in which cleavage polyembryony follows the same pattern.

Swamy (1943) reported three different modes of cleavage other than the earlier mentioned in *Eulophia epidendraea* (fig 8.3).

a. Development of additional embryo takes place from the mass of cells formed by the irregular division of the zygote. From this irregular mass of cells some cells at the chalazal end develop into the embryo.

b. The proembryo some time gives rise to small bud like out growths which develop into embryos.
c. The proembryo becomes branched and additional embryo is formed at the tip of each branch.



(A) (B)

Fig 8.3: A&B. cleavage polyembryony in Eulophia epidendraea

2. Origin of embryo from cells of the embryo sac other than the egg

In this type of polyembryony, the additional embryo may develop from the cells of either synergid or antipodal of the embryo sac. In this type the most common cell contributing to the embryo is synergid. Further, depending upon the nature (fertilized or unfertilized) of synergids , the embryo may be diploid or haploid. In *Sagittaria graminea, Aristolochia bacteata, Crepis capillaries* and *Poa alpine,* one or sometimes both synergids may get fertilized due to the entry of more than one pollen tube in the embryo sac or presence of additional sperms in the same pollen tube, hence along with the zygotic embryo synergid embryo is also diploid in nature. But in *Phaseolus vulgaris* and *Argmone mexicana,* the synergids remain unfertilized and hence the embryos are haploid in nature.

Formation of embryo form the antipodal cells are of very rare occurrence. But, such embryos are reported from *Ulmus glabra* (fig 8.4)*, Allium odonum, Sedum gabaria* and *Paspalum scrobiculatum.* Formed embryos generally fail to grow into mature embryo and no reports for antipodal forming germinable embryos is on record. Besides this, the only example of *Brachiaria setigera* is on record in which the endospermic cells contributing to the embryo formation, otherwise endosperm forming embryos are in doubt.

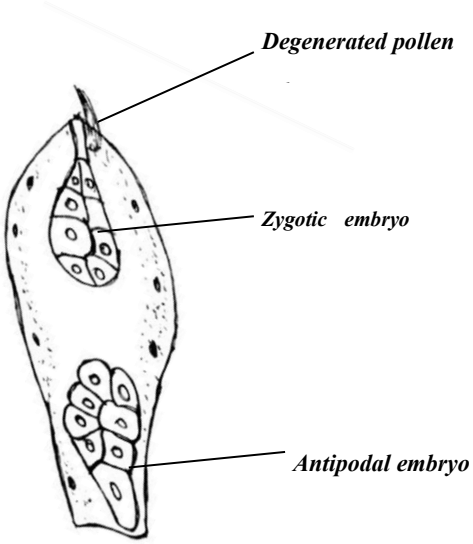


Fig 8.4: Development of embryo from antipodal cells along with the zygotic embryo in Ulmus

3. Development of embryo from the additional embryo sac in the ovule

Presence of multiple or additional embryo sacs in the same ovule, further coupled with the fertilization of their eggs results in the formation of extra embryos (polyembryony condition) in the seed. This type of polyembryony may arise in the following ways:

(1) Due to differentiation of more than one MMC in the same ovule(*Solanum melongena, casuarinas montana, Casuarina equisetifolia, Hydrilla verticellat*)
(2) By activation of two or more derivatives of the four megaspores formed during the reduction division of the MMC (*Rosa*).
(3) From the cells of nucellus and integument (*Mangifera, Opuntia, Limnanthes*).

8.4.3 Induced polyembryony

The embryo arising from the material outside the embryo sac (nucellus and integument) are called adventive embryos. In nature polyembryony arises spontaneously and it was believed in past that a specific physical and chemical environment is required for the development of embryo which is present only in the embryo sac. But with the time it was established by number of workers that not only the ovular tissue but all the cells of the plant are capable of forming the embryos, which may also be generated *in-vitro* by providing necessary nutritional and environmental conditions. The embryo produced from the *in-vitro* culture under the influence of different growth regulators is known as somatic embryo, which is one of the common practices of generating plantlet in plant tissue culture exercises. In some cases, the freshly harvested seeds are treated with the growth regulators (NAA, 2,4-D, TDZ) at low concentrations which may induce many abnormalities resulting in the development of twin embryos in some seeds.

8.4.4 Cause of polyembryony

To explain the occurrence of polyembryony in the seeds, several theories have been given but none of them sufficiently validate their assumptions.

1. Necrohormone theory

This theory was proposed by Haberlandt (1921, 1922) and according to him, the degeneration of cells of the nucellus act as source of stimulus for the adjacent cells to divide and form the adventive embryo. To support his assumption, he attempted to induce the polyembryony condition in the *Oenothera* plant by damaging its cells with the help of needle and he successfully got two embryos in ovule which were considered to be the nucellar origin. But by repeating his results to other plants many workers reported negative results.

2. Recessive gene theory

Leroy (1947) considerd that in *Mangifera indica* polyembryony was caused by one or more recessive genes. In support of his view he stated that the mango in its primary center of origin have dominant genes thus only monoembryonate forms occur, while in secondary centre of origin have forms with recessive genes and the mango in these areas have polyembryonate seeds. But this concept failed to hold its validity because in some varieties of Indian mangos, the polyembryonate condition is also recorded.

Some other workers explained the causes of polyembryony in their own ways, According to them the monoembryonate condition in some *Citrus* plants is due to the presence of certain volatile and non-volatile embryogenic inhibitors in ovule which are absent in the polyembryonate ovules.

8.5 PARTHENOCARPY

Parthenocarpy, the term introduced by F. Noll in 1902, designate to the formation of fruits without pollination. In nature, successful transfer of pollen grain from the anther to the stigma is essential and first step towards the process of fertilization, in which one of the male gamete fuses with egg forming zygote. Fertilization results in the development of fruit from ovary and seed from ovule. But in nature some fruits develop without fertilization i.e., fruiting without the union of male and female gametes. Thus, they are not viable for germination. Parthenocarpy is often common in the species which generally have large number of ovules inside fruits for example, fig, tomato, cucumber etc.

8.5.1 Types of Parthenocarpy

(i) **Genetic parthenocarpy**: Genetic parthenocarpy is reported in many edible fruits, even the same species have both seeded or parthenocarpic fruits. Such types of varieties are generally produced as a result of mutation or hybridization. Fig, tomato, oranges, banana etc are some classical examples of plants that show genetic parthenocarpy.

(ii) **Environmental parthenocarpy**: Parthenocarpic fruit formation is also forced by unfavorable weather conditions (temperature, frost, fog). In *Solanum melongena* as the plants grow older and winter approaches, some time they start producing seedless fruits instead of seeded fruits, same is also reported in Tobacco. Cucumber, Olive and Pear are some other examples in which temperature, fog and frost are known to induce parthenocarpy.

(iii) **Chemical or induced parthenocarpy**: Chemicals responsible for parthenocarpy is suggested by Gustafson (1939), he reported that auxin content in the ovaries of parthenocarpic species of oranges and grapes is higher than the non-parthenocarpic species and proposed a hypothesis that higher concentration of auxin in the ovaries is responsible for the development of parthenocarpic fruits. Gibberellin concentration increases after fertilization and utilizing this concept, artificial application of gibberellins was practiced in producing parthenocarpy fruit.

(iv) **Genetically engineered parthenocarpy**: Rotino *et al.* (1997) successfully developed genetically engineered parthenocarpy varieties of egg plant and tobacco plant by inserting the chimeric gene DefH9-iaaM. The iaaM gene is responsible for the increased synthesis of auxin in the tissues and organs of the transgenic plant, while DefH9 acts as the ovule specific promoter and regulatory gene regulates the expression of iaaM only in the ovules without affecting vegetative growth. Further, the parthenocarpy character is transmitted to the progeny in a Mendelian fashion as dominant trait.

8.5.2 Advantage of Parthenocarpy:

Parthenocarpic fruits are of high demand in the market because seedless fruits are more preferred over the seeded fruits. Further, seedless fruits are ideal for the industrial processing such as preparing jam, juice etc. and besides this parthenocarpy increases the edible proportion of the fruit.

8.6 SUMMARY

The normal sexual reproduction involves two important characteristics i.e., (i) Meiosis, in which a diploid cells transform into a haploid cells which may be male or female gametes. (ii) Syngamy, in which two haploid gametes of opposite sex fuse and restore the diploid stage.

In this unit we have learned about the different aspects of embryology and seed formation. In general in an ovule there is one embryo. The mature embryo sac consist of an egg apparatus (two synergids and one egg) at the micropylar end, three antipodals at chalazal

end and two polar nuclei at the center of the embryo sac. During the course of fertilization, egg fuses with one of the male nuclei to form zygote which after embryogenesis gives rise to embryo and at the same time second male nucleus fuses with the secondary polar nuclei giving rise to endosperm. In general, after fertilization one embryo is formed per embryo sac but several time occurrence of more than one embryo in the same embryo sac is also found and this situation is called as polyembryony. First reported in *Citrus* by Leeuwenhoek. On the basis of origin this extra embryo may be gametophytic or sporophytic. Further, additional embryo may develop from the cells of synergid, cleavage of embryo, antipodal or adventitiously. Polyembryony may also be of spontaneous nature or experimentally induced. Besides this, in this unit we have discussed about the apomixis, which literally mean formation of seed without fertilization (asexual means). In apomixis seeds are formed without fertilization and is a common event in several angiospermic families (Rosaceae, Rutaceae, Asteraceae) . Further, two different types of apomixis is reported i.e., gametophytic (female gametophytic origin) and sporophytic (directly from the diploid sporophytic cells of the ovule). Apomixis is in high demand in crop industry because apomixis is very useful for the fixation of heterosis, production of pure heterozygous line and others. Further, formation of fruit is also post fertilization event and fertilized ovary transforms into fruit, but in several cases fruits are developing without fertilization of ovule and thus fruits are without seeds and this phenomenon is called as parthenocarpy which again is of horticultural interest because of production of fruits without seeds.

8.7 GLOSSARY

Apomixis: Phenomenon of producing asexual seeds in the absence of sexual fusion between gametes.

Parthenocarpy: The term designate to the development of fruits without seeds.

Syngamy: Syngamy means fusion of gametes.

Stenospermocarpy: Stenospermocarpy is the biological mechanism that produces seedlessness in some fruits**.**

Endosperm: The endosperm is the nutritive tissue produced as a result of triple fusion inside the seed of most of the flowering plants

Polyembryony: The phenomenon of occurrence of more than one embryo in the seed.

Embryo: Plant embryo is a future plant formed from the zygote and forms a connecting link between present generation and the future generation.

Seed: Seed is a ripened ovule which contains the embryo or the miniature of plant body.

8.8 SELF ASSESSMENT QUESTIONS

8.8.1. Short answer type questions.

Q.1. What is apomixis ?
Q.2. Write short note on adventive embryony.
Q.3. Write short note on cleavage polyembryony.
Q.4. What is Parthenocarpy ?
Q.5. Write short note on significance of parthenocarpy.
Q.6. Differentiate between Seedless and parthenocarpic fruit.

8.8.2 Multiple choice questions:

1. Polyembryony was first reported by
a. Leeuwenhoek b. Springle
c. Strasburger d. Bauhin

2. Embryo of antipodal origin have been observed in
a. Orchids b. *Mangifera*
c. *Optunia* d. *Ulmus*

3. Development of fruit without fertilization is
a. Parthenogenesis c. Parthenocarpy
b. Apomixis d. none of the above

4. Polyembryony is commonly observed in
a. *Citrus* b. Banana
c. Mangifera d. Tomato

5. Bananas are seedless because they
a. Reproduce asexually b. Are triploid
c. Are sprayed with hormone d. Are parthenocarpic

6. Seedless grapes are produced due to
a. Parthenocarpy b. Crossing over
c. Parthenogenesis d. None of these

7. In non-recurrent agamospermy the embryo is
a. Nucellar c. Haploid
b. Integumental d. Diploid

8. Embryo develops from nucellus and integument is known as

a. apospory b. apogamy
c. apomixes d. adventive embryony

9. Apomixis is
a. Development of plant in darkness b. Development of plants without fusion of gametes
c. Inability to perceive stimulus for flowering d. Effect of low temperature on plant growth

10. Diplospory is development of embryo from
a. Nucellus b. Megaspore mother cell
c. Integument d. Megaspore

8.8.2-Answers

1. (a); 2. (d); 3. (c); 4. (a); 5. (d); 6.(a) 7.(c); 8. (d); 9. (b); 10. (b).

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8.11 TERMINAL QUESTIONS

Q.1. Describe in brief about the parthenocarpy and its types and practical importance in Horticulture.

Q.2. Define Polyembryony? Its different types and practical applications.

Q.3. Explain different type of apomixis reported in Angiosperms.

UNIT-9: PLANT TISSUE, ANTHER AND EMBRYO CULTURE AND EMBRYO RESCUE

9.1OBJECTIVES

After reading this unit students will be able to-

- Know about the different culture media and their chemical compositions.
- Understand the concept of sterilization and different approaches for sterilization.
- Understand the techniques of haploid production via anther culture.
- Understand the techniques of embryo culture.
- Understand the need of embryo rescue.
- Applications of plant tissue culture in embryo rescue.

9.2 INTRODUCTION

The science of plant tissue culture is essential component of Biotechnology with potential for serving crop improvement programs. Technique is based on the work of Haberlandt (1902) who successfully cultured the carrot pith on the nutrient medium although he himself was neither successful to regenerate plantlets nor his cultured cells divided in the culture medium. But the art of growing plants under total aseptic and artificial conditions have grown into an industry. With this technique micropropagation of rare and novel genotypes, production of haploid plants, raising of disease free plants and production of secondary metabolites have been achieved. It was Morel (1960) who successfully cultured a virus contaminated orchid by culturing its apical part to regenerate complete virus free plantlets. Formulation of nutrient medium started as soon as after realization that the need of single medium is not satisfactory for the growth of all tissues. The impressive array of basic nutrient media came with the formulation of number of culture media which differ only in narrow limits mostly in the concentration of individual ingredients. A basic balanced medium came with the formulation of White (1934, 1954), Murashige and Skoog (1962) and Gamborg (1968).

In vitro technique of anther culture, appeared with the work of Guha and Maheshwari (1964). The technique of haploid production has been extended successfully to numerous other plant species. To-date, 'anther androgenesis', has been reported in over 134 species and hybrids distributed within 25 families. Embryo culture is a method of cultivation of zygotic embryos excised from ovules. The main reason for this is that the embryo culture technique allows the investigator to manipulate the embryo experimentally outside the ovule or the seed in ways that are not easy to accomplish when the embryo is enclosed in the ovule or seed.

Plant tissue culture offers one of the best alternatives for mass conservation of threatened and endangered plants and holds great promise to plant breeders, pharmaceutical industries and others.

9.3 GENERAL TECHNIQUE OF PLANT TISSUE CULTURE

9.3.1Culture medium

Culture medium, backbone of organogenesis is composition of mineral salts, carbon source, vitamins, growth regulators and other organic supplements required by plants for their growth and metabolism. Generally all culture media are chemically defined. Since the appropriate compositions of culture medium largely determines the success of the *in vitro* cultures. A variety of culture media have been developed in past. The chemical compositions of some important culture media are listed in Table 9.1.

Table 9.1. Composition of different plant tissue culture media.

S. No	Components	White's	MS	B5
1	Ammonium nitrate (NH_4NO_3)	-	1650 mg	-
2	Potassium nitrate (KNO_3)	80 mg	1900 mg	2527.5 mg
3	Calcium chloride ($CaCl_2 \cdot 2H_2O$)	-	440 mg	150 mg
4	Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	750 mg	370 mg	246.5 mg
5	Potassium phosphate (KH_2PO_4)	-	170 mg	-
6	Potassium chloride (KCl)	65 mg	-	-
7	$NaH_2PO_4.H_2O$	16.5 mg	-	150 mg
8	Na_2SO_4	200 mg	-	-
9	$Ca(NO_3)_2.4H_2O$	-	300	-
10	$(NH_4)_2SO_4$	-	-	134 mg
11	$MnSO_4 \cdot H_2O$	-	-	10 mg
12	Boric acid (H_3BO_3)	1.5 mg	6.20 mg	3 mg
13	Manganese sulphate ($MnSO_4 \cdot 4H_2O$)	7 mg	22.3 mg	-
14	Zinc sulphate ($ZnSO_4 \cdot 7H_2O$)	3 mg	8.6 mg	2 mg
15	Potassium iodide (KI)	0.75 mg	0.83 mg	0.75 mg
16	Sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$)	-	0.25 mg	0.25 mg
17	Cupric sulphate ($CuSO_4 \cdot 5H_2O$)	0.01 mg	0.025 mg	0.025 mg
18	Cobalt chloride ($CoCl_2 \cdot 6H_2O$)	-	0.025 mg	0.025 mg

19	Ferrous sulphate ($FeSO_4 \cdot 7\ H_2O$)	-	27.85 mg	-
20	EDTA –Na ferric salt	-	-	43 mg
21	$Na_2EDTA \cdot 2H_2O$	-	37.35 mg	-
22	Sequestrene 330 Fe	-	-	28 mg
Common Organic Additives mgl^{-1}				
23	Myo-Inositol	-	100 mg	100 mg
24	Pyridoxine HCL	0.01 mg	0.5 mg	1 mg
25	Thiamine HCL	0.01 mg	0.1 mg	10 mg
26	Glycine	3.0 mg	2.0 mg	-
27	Nicotinic acid	0.05 mg	0.5 mg	1 mg
28	Cysteine	1 mg	-	10 mg
29	Sucrose	20,000 mg	30,000 mg	20,000 mg
Gelling Agent gml^{-1}				
30	Agar (6-8 gm)	8 gm	8 gm	8 gm

9.3.1.1 Inorganic Nutrients: Mineral elements are very important in the life of a plant. Besides, C, H, and O, there are some 12 other elements or essential elements (N, P, K, Ca, S, Mg, Fe, Zn, Mn, Cu, B and Mo) known for the plant growth and synthetic nutrient medium have all these in chemical form. The essential elements further divided into Macro elements (N, P, K, Ca, S and Mg) and according to recommendations of International Association for Plant Physiology these elements are required in concentration greater than > 0.5 m mol l^{-1}, while micro elements (Zn, Mn, Cu, B and Mo) are required in trace amount or concentration lesser than < 0.5 m mol l^{-1}. They are so essential that plant cannot complete its growth without these for example calcium is a component of cell wall and acts in cell signalling, Nitrogen is an important component of nucleic acid and amino acids, Potassium regulates osmotic potential and acts as principal inorganic cation, like some other important enzyme cofactors, Mn, Zn, Mo, Cu etc., Magnesium besides acting as an enzyme cofactor is component of chlorophyll. Iron is usually provided as chelate with EDTA, in this form iron remains available upto pH 8.0. Further, iron EDTA complex allows the slow and continuous release of iron into the culture medium.

9.3.1.2 Carbon source and Hexitols: Green cells or tissue, callus etc, are generally not phytosynthetically active thus require carbon source. Sucrose in the range of (20-50gm l^{-1}) are widely used in all plant tissue culture media. Besides sucrose, glucose, fructose and other carbohydrates such as lactose, maltose, starch and galactose are also used but proved inferior than the sucrose. But in some monocots glucose proved superior over sucrose.

Myo-inositol has been found to be an important carbohydrate, which improves cell growth. Myo-inositol involved in cyclitol biosynthesis, germination of seeds, sugar transport,

mineral nutrition, membrane structure, hormonal homeostasis and stress physiology (Loewus and Loewus, 1983).

9.3.1.3. Growth regulators: Hormones are organic compounds naturally synthesized in plants having pleiotropic effects in the plant's life cycle. Hence, involved in the wide spectra of the development and physiological processes. The plant growth regulators used in plant tissue culture are generally classified into five types i.e., Auxin, Cytokinin, Gibberellins, Ethylene and Abscisic acid. Of all, auxin and cytokinin are of primary importance. Auxins (IAA, IBA, NAA, 2,4-D, Dicamba, NOA and Picloram) are commonly used to support or promote cell growth and cell division. Auxins are well known to form callus and induce root in *in-vitro* shoots. 2,4-D is widely used for callus induction. Cytokinins (Zeatin, BAP and Kinetin) employed in the culture medium to promote cell division; they are derivatives of Adenine and have potential role in shoot organogenesis and morphogenesis. Abscisic acid (ABA) is a thermal stable but light sensitive growth regulator used to produce Somatic embryos and useful in embryo culture. Gibberellins about 20 in number, but GA_3 is most preferred growth regulator and generally used for stem elongation, due to its inhibitory effect on callus growth induced by auxin this hormones is not frequently used in plant tissue culture studies.

9.3.1.4. Vitamins: Under natural germination conditions, plants synthesize vitamins required for growth and development. Vitamins have catalytic functions in enzyme reactions, B_1 (Thiamine) vitamin is considered as essential for *in vitro* plant tissue culture, while other vitamins such as Pyridoxine (B_6), Nicotinic acid (B_3) are added to culture media to enhance growth.

9.3.1.5. Gelling agents: Gelling agents are generally added into the culture medium to solidify it. Agar, a sea weed is most popular solidifying agent in regular experiments, since agar is a natural and neutral product and the chance of impurities are always there so high quality agar should be used. Gelrite a transparent gelling agent produced by fermentation of *Pseudomona*s species is also used as gelling agent.

9.3.2- Sterilization

Plant tissue culture is an aseptic technique of culturing cells or tissues to raise complete plantlets. Sterilization is of pre-requisite and sterilization is a procedure used for elimination of microorganisms from all materials being used in experiment. Methods of sterilization for different materials are:

(i) Dry heat sterilization: This method of sterilization involves the use of dry heat (160-180 ^{0}C) for 2-3 hours; this exposure is regarded as equivalent to steam sterilization. Materials to be sterilized by this method include glasswares, forceps, needles etc.

(ii) Steam sterilization or autoclaving: Autoclaving includes sterilization at 121 ^{0}C at 15 psi at different time durations, depending on materials to be sterilized (Fig 9.1). Culture medium, autoclavable plastic bags, micropipettes, glasswares and contaminated cultures.

(iii) Flame sterilization: Instruments like scalpels, forceps, needles are flame sterilized during work.

(iv) Filter sterilization: All heat labile chemicals (growth regulators, vitamins) are filter sterilized by passing the solution through a membrane filter of 0.45 μ or lower pore size.

(v) Surface sterilization: Plant materials to be used for plant tissue culture practices are generally full of contaminants, so in order to get material free from contamination, different of chemicals are used. This method involves the eradication of microorganisms with the help of chemicals. Some of the commonly used chemicals are sodium hypochloride, mercuric chloride, calcium hypochloride, silver nitrate, hydrogen peroxide, bromine water etc.

(vi) Wiping with alcohol: The surface of growth chambers, platform of the laminar air flow etc are generally sterilized by wiping them with 70-80% alcohol.

Fig 9.1: Autoclave

Inoculation: Inoculation is conducted in the transfer chamber which may be an ordinary bacteriological glove box made of fibre glass or plexiglass fitted with accessories or more sophisticated Laminar air flow chamber (Fig 9.2). The laminar air chamber provides a cabinet with continuous air flow system providing cabinet for inoculation work as well as tissue transfer procedures.

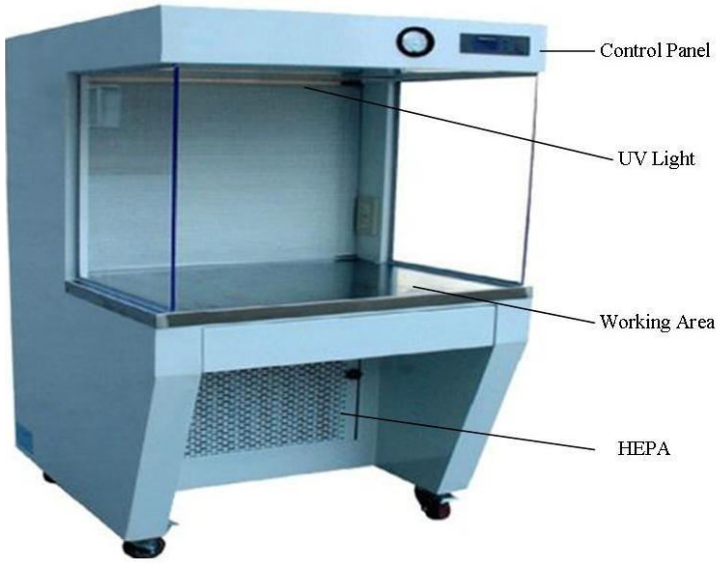

Fig 9.2: Laminar Air Flow Chamber

9.4 ANTHER CULTURE

9.4.1 Concept and Pathway of Development

Anther culture is a technique of culturing anther/pollens under aseptic *in vitro* conditions to raise haploid plantlets. The purpose of anther and pollen culture is the production of haploid plants as the significance of haploids has been realized for a long time.

Plants breeders are interested in haploid plants because either spontaneous doubling or an application of the chemical colchicine to double the chromosome number gives rise to homozygous plant. In this technique, the immature pollen is made to divide and grow into callus or embryonic tissue to produce haploids either through organogenesis or embryogenesis.

The plant for anther culture should be grown under ideal conditions i.e., controlled temperature, light and humidity, this foster the experimental setup by providing more vigor and less contaminated explant. Further, since the age of the plant also have its role in anther culture it is always advocated to have anther from a young plant. As abnormalities appears with the age and *in vitro* responses decline. The selected buds for culture are surface sterilized with a suitable disinfectant and aseptically placed on a sterilized culture medium. Dealing with plants having minute flowers, such as *Asparagus* and *Trifolium,* only the perianth may be removed and the rest of the bud with the stamens intact inoculated. The gap between bud collection and anther/pollen culture should not exceed 2 hrs. After inoculation, the wall of tissue gradually starts turning brown and calli or pollen embryos start appearing in responsive anthers within few weeks. The generated embryo sometime

require to transfer to new medium (shooting or rooting medium) for further growth or some time grow on the same medium depending upon plant species to species. The regenerated plantlets then acclimatized in soil conditions before transferring to the green house.

In vitro androgenesis for the production of haploid plants are grouped into two different modes.

(i) Direct androgenesis: In direct androgenesis, the microspores behave like a zygotes and undergo changes to form embryoids which ultimately give rise to a plantlets.

(ii) Indirect androgenesis: In contrast to the direct androgenesis, the microspores, instead of undergoing embryogenesis, divide repeatedly to form a callus which differentiates into haploid plantlets.

Callus formation in isolated pollen culture of *Brassica oleracea* and the hybrid (*B. oleracea x B. alboglabra)* was first reported by Kameya and Hinata.

Since then the technique of pollen culture has been considerably improved and androgenic plants through isolated pollen cultures have been raised for many crop plants, including *Brassica carinata, B. campestris*, *B. napus, B. nigra, B. oleracea*, *B. rapa*, *Hordeum vulgare*, *Oryza sativa*, *Petunia*, *Nicotiana rustica, N. tabacum*, *Triticum aestivum* and *Zea mays*.

Pathway of Development

Based on the few initial divisions in the microspore, five different modes of *in vitro* androgenesis have been identified (fig: 9.3).

(i) ***Pathway I***. In some cases the uninucleate pollen divides unequally to larger vegetative and smaller generative cells. In vegetative cell further division leads to the formation of callus/embryo from which the new sporophyte or plantlet arises. The generative cell in these cases either degenerate immediately or after few mitotic divisions e.g., *Triticum aestivum*, *Nicotiana tobacum*, *Hordeum vulgare*, *Triticale* and *Capsicum annuum*.

(ii) ***Pathway II***. In *Datura innoxia* the microspores divide symmetrical and the two identical daughter cells contribute to the sporophyte development.

(iii) ***Pathway III***. In contrast to pathway II, in *Hyoscyamus niger,* the generative cell alone is responsible for the formation of the predominantly pollen embryos. The vegetative cell in this pathway either does not divide or does so only to a limited extent forming a suspensor like structure.

(iv) ***Pathway IV***. As in pathway II, vegetative and generative cells are formed but in this case both the cells divide further and participate in the development of the sporophyte. This mode of androgenesis has been reported in *Datura innoxia*. However, Sunderland and Dunwell (1974) are of the opinion that this pathway might be operative in other plants which form high frequency non-haploids, such as *Datura metel* and *Atropa belladonna*.

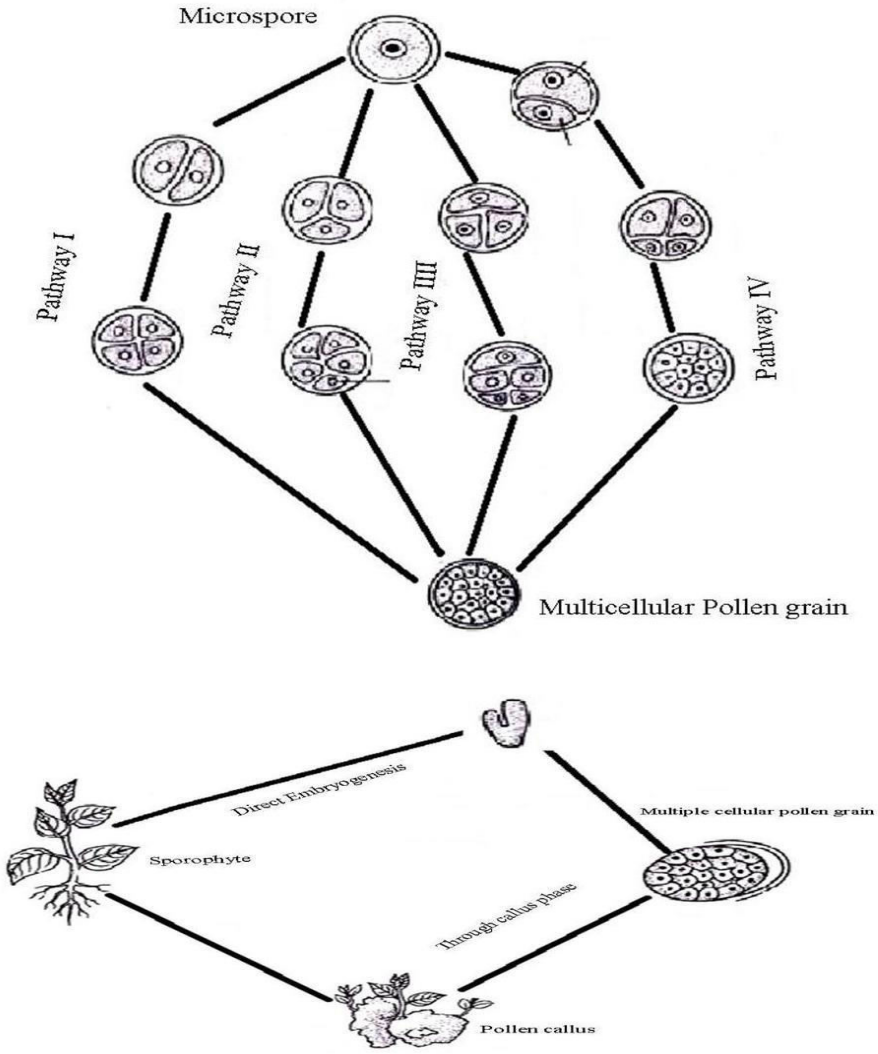

Fig 9.3: Pathway of Pollen development

9.4.2 Later developments

Irrespective of the pathway followed during the early divisions, the inoculated pollen grains in suitable culture medium become multicellular and burst open to release the tissue (callus/embryo). In species like *Arabidopsis*, *Triticale, Asparagus*, pollen grains after several divisions liberate in the form of the multicellular mass of cells forming a parenchymatous callus. This callus then develops shoot and root through organogenesis and thus developing into a plantlet as in other plant tissue culture practices. Besides this, several species like *Datura, Atropa, Hyoscyamus, Nicotiana* etc., the pollens after several divisions burst into multicellular mass which assumes to form embryo (globular embryo) directly which undergoes to normal embryogenic stages (heart-shaped, torpedo-shaped, and cotyledonary stage) to yield haploid plant. Further there are several reports on record confirming the manipulation of the route for their development by changing the medium compositions e.g., *Oryza sativa* and *Hyoscyamus niger* in both cases the composition of the

medium affects their mode for the development either by embryo formation or through callus formation into the final haploid plant production.

9.4.3 Factors Affecting Androgenesis

Physiological status of the donor plants

Physiology of the donor plants affects the *in vitro* responses in number of ways:

1. The buds for the *in vitro* responses, collected from the first flush of flowers show better androgenic response than those borne later.
2. The anthers excised towards the end of the flowering season respond inferior than those collected earlier. But in *Brassica rapa,* pollen grains from old, sickly looking plants produced more embryos than those from young and healthy plants.
3. Stresses such as water stress and nutrient stress also have their prominent role in androgenesis and number of reports suggested their promotory role in androgenesis.
4. Chemical treatment of the donor plant with etherel, auxin, gametocidal and anti-gibberellins provide more responsive anther than those of untreated e.g. Rice, Wheat etc.
5. Growing plants under lower temperature improved the yield of pollen embryos in some plants e.g., *B. napus.*

Pre-treatment of cultured anthers

Treatment of cultured anther with certain physical (temperature, light) and chemical agents prior to their transfer to culture room conditions has proved promotory for *in vitro* androgenesis.

(a) *Temperature shock*. Cold and Hot treatment to buds or cultured anther is known to promote androgenesis in many species. For example, in *Nicotiana tobacum* responses increased upto 58%, when the buds were pre-treated at 5ºC for 72 hrs as against 21% in control. Besides this, high temperature shock is known to improve results in *Capsicum* and some genotypes of wheat.

(b) *Centrifugation*. In number of plants centrifugation of anther at different density proved to enhance the percentage of androgenic anthers. In *Datura innoxia* the centrifugation of anthers at 40 g for 5 min after cold treatment of buds at 3^0C for 48 h improved the percentage of androgenic anthers.

(c) *γ-Irradiation*. Promotory effect of ***γ*** –irradiation treatment to androgenesis has been reported in number of reports. Low dose treatment of ***γ***- irradiation has been reported to promote pollen callusing and pollen embryogenesis in *B. napus*. The *in vitro* response for androgenesis has been improved by three fold, when the buds are young. Besides this, old

buds which normally do not exhibit androgenesis, became responsive after γ -irradiation. Irradiation is known to inactivate nuclei and alter the levels of auxins and cytokinins in the tissues. These actions may be involved in the promotion of androgenesis by low irradiation.

Culture medium

Culture medium and their compositions have direct effects on *in vitro* responses. In general, two mitotic divisions occur in a microspore, but for androgenesis, microspores have to perform number of divisions. These additional divisions are under the control of composition of culture medium coupled with growth regulators.

1. Addition of ethereal in basic culture medium has been found to enhance androgenic response in anther cultures of tobacco.

2. Humid temperature also fosters the multiple divisions in the pollen grains of datura and tobacco.

3. Sucrose also known to have promotory effect on multiple divisions required for androgenesis in wheat, potato and *Datura*.

4. Iron in culture medium also have its crucial role in androgenesis, medium lacking it or containing it below the threshold concentration of Fe.EDTA, the development of embryo has been reported to be arrested at the globular stage. Besides this, the use of iron is most effective when supplied for post inductive development of pollen embryo.

5. Culture medium having low nitrogen particularly ammonium nitrate promoted androgenesis.

6. Addition of 2,4-D, ascorbic acid, activated charcoal (AC), glutathione, and glucose in place of sucrose in the basal culture medium has improved androgenesis and found stimulatory for androgenesis in different species.

Effect of gaseous environment in culture tubes

Gaseous compositions of culture tubes or flasks also have an effect on androgenesis. Removal of CO_2 from the culture tubes by KOH resulted in a decline in androgenesis, which might be due to the inhibition of ethylene by CO_2. At the same time elevated concentration of CO_2 (2%) almost doubled the androgenic response in *Lolium perenne.*

Effect of light

Effect of light does not seem to have inhibitory or promotory effect on androgenesis but in some plants (*Datura innoxia, Annona squamosa* and *Nicotina tabacum*) initial incubation of culture in dark promoted androgensis.

9.4.5 Applications

1. Production of pure homozygous lines of the cross pollinating species and hybrids are highly desirable to increase the efficiency of selection and production of homozygous plants. The conventional plant breeding methods to produce homozygous plants is laborious, lengthy requiring 7-8 recurrent cycles of inbreeding. while homozygous plants can be obtained in a single generation by diplodization of the haploids produced by androgenesis.

2. Pure homozygous line can be produced by haploid production in all those plants, where homozygous line production is impractical due to self-incompatible and male sterility of the plants.

3. Haploids are also extremely useful for detecting recessive mutants which may not express themselves in the heterozygous diploid background.

4. Androgenesis helps in recognizing gametophytic variations produced by recombination and segregation during meiosis.

9.5 EMBRYO CULTURE AND EMBRYO RESCUE

9.5.1 Concept of Embryo Culture and Embryo Rescue

Embryo culture represents the earliest technique to obtain viable offsprings by culturing a young embryo from seed on to suitable culture medium to regenerate to complete plantlet. The technique represents an important milestone in efforts to identify the requirements essential for continued growth, differentiation, and morphogenesis of embryos and allows the investigator to manipulate the embryo experimentally outside the ovule or the seed in ways that are not easy to accomplish when the embryo is enclosed in the ovule or seed.

The first systematic attempt to grow the embryos of angiosperms *in vitro* under aseptic conditions was made by Hannig (1904) who cultured mature embryos of two crucifers, *Cochleria* and *Raphanus.*

Based on the number of reports on behaviour of cultured embryos belonging to Asteraceae, Brassicaceae, Solanaceae, Polygonaceae, Cucurbitaceae, and Poaceae, Dieterich (1924), pointed out two important generalizations which have been proved to be significant in understanding the physiology of the growth of the embryos. One was that the embryo grown *in vitro* usually skips a resting period that is observed when it is part of the intact seed. In addition to this it was found that a solid medium containing Knop's mineral salts and 2.5-5.0% sucrose can support normal growth of isolated embryos from mature seeds but in the same medium embryos from immature seeds tend to form malformed seedlings, omitting the regular stages of embryogenesis, came to be known as 'precocious germination' in embryo culture studies.

Embryo rescue literally means to rescue an embryo and regenerate the embryo into a viable plant *in vitro* using plant tissue culture technique which otherwise is going to be aborted with the time due to several physiological or biological reasons.

In the literature the term embryo culture is often used synonymously with embryo rescue. But to be accurate, embryo rescue means the culturing the embryo to generate a plantlet which under normal conditions would not develop naturally.

The principle of embryo rescue or embryo culture technique is the aseptic isolation of embryo and its transfer to a suitable medium for development under optimum culture conditions.

Hybrid plants could be obtained by culturing of immature embryos, a feat considered impossible a decade ago. But with the refinement in techniques and advancements in knowledge of the nutritional aspects of cultured tissue, it has now been possible to achieve success with a large number of wide crosses among the cereals (barley × rye or barley × wheat etc).

Number of workers reported that embryos of non-viable hybrids possess the potential for initiating development but are inhibited from reaching adult size with normal differentiation.

Embryo can be rescued by two different approaches

1. The embryos are isolated and transplanted into foreign endosperm as for instance, wheat or rye endosperm with a view of raising wheat plants.
2. The embryos are isolated aseptically and then cultured on suitable culture medium.

The excised embryos are then placed in suitable culture medium by different approaches. Three important approaches are:

1. **Direct method**: Young embryo placed directly on culture medium.
2. **Embryo nurse endosperm technique**: This technique is usually used to rescue the very young embryo. This technique uses the already *in vitro* cultured endosperm as a base for implanting the rescuing embryo.
3. **Culture of pollinated ovule**: this technique is used for all those interspecific crosses where flower drops before the embryo reaches the minimum size required for excising the embryo from the seeds. So in these cases pollinated ovule or some time ovary may be cultured for a period till the embryo reaches a minimum size (*G. arboreum* × *G. hirsutum*).

9.5.2 Factors affecting to embryo culture and embryo rescue

In distant crosses, seeds are generally sterile or produce no seeds due to the pre fertilization barriers. These include all factors that hinder pollen tube growth by the stigma or upper style. Post- fertilization barriers hinder or retard the development of zygote after fertilization and normal development of the seed. If the seed is produced, the endosperm of

the seed collapses. This collapsing of the endosperm of growing seeds is due to incompatibility between maternal tissue and the endosperm. The young embryo ceases to grow and abort (pre-heart or 4-8 celled stages). Endospermic collapse often leads to endosperm disintegration and consequent disruption of kinetin supply which could be overcome by embryo culture.

The most important aspect of the embryo culture is the selection of suitable medium necessary to sustain continued growth of embryo to mature. When the rescuing embryo is younger or undifferentiated, embryos require higher concentration of sucrose (12-18%) as suggested by number of reports but sucrose requirement of embryo declines sharply with embryo growth and embryos need to be transferred to new medium with normal or low level of sucrose for better results. Further, in few reports it was evident that immature embryos grew best on medium having reduced nitrogen source such as glutamine, casine or a mixture of amino acids and B- vitamin. Plant extracts such as *Ginkgo* endosperm were conducive to the growth of *Ginkgo* embryos. After few weeks the embryo must be transferred to a normal basal medium for their proper growth and further organogenesis. This particular choice of medium for their maturation is called as "Embryo factor". Sometimes other complexes such as coconut milk and plants extract may also be proved beneficial or foster the results of embryo culture.

A phenomenon that is some time encountered in embryo culture is the precocious germination which is the instinct of young embryos skipping development into full term embryos. This results in the development of malformed seedlings. Addition of GA_3 and Kn induce anomalous growth. These abnormalities could be diminished to great extent by exposing the *in vitro* cultured immature embryos to high illumination, moderately high temperature and reduced Oxygen tension. Anomalous growth due to GA_3 and Kn can be checked by using ABA as it seems to play a regulatory role in embryo during successive stages of development and bestows the ability of embryo to grow into adult. Precocious germination also checked by placing some ovular tissue external to embryo, which is generally not synthesized by younger embryo. Higher concentration of auxin also results in the callus formation from the immature embryo, but moderate to low concentration favours the growth of immature embryo to adult.

9.5.3- Application of Embryo culture and Embryo rescue

1. **Overcoming Seed dormancy:** Seed dormancy, the term means the failure of seeds to germinate although environmental conditions including water, temperature, light and gases are favourable for germination or the incapability of a viable seed to germinate under favorable conditions. Some time seeds may have some inhibitors in endosperm (*Iris* stable chemical inhibitor in the endosperm) or seed coat. These factors may be circumvented by embryo excision and culture the embryo *in vitro*. Further this technique is very beneficial to produce viable seedlings in those species whose seeds do not germinate in nature (*Musa balbisiana* and *Colocasia*).

2. **Prevention of embryo abortion in early ripening fruits:** In many early ripening varieties such as cherry, apricot, plum, produced seeds are not able to germinate and dies under the soil due to incomplete embryo development. Due to this incomplete development of embryo, abortion generally resulted which is due to the aborted connection of water and nutrient from endosperm. So embryo culture has been used to culture these types of plants.
3. **Regeneration of plants in hybrids:** Distant crosses fail due to several reasons, but when embryo fails to develop due to endosperm factor, embryo culture technique may used to rescue the embryo.
4. **Seed germination:** Some time seed of plant require obligatory parasites without which the germination of seeds becomes impossible *in vivo*, but this technique foster the germination of the seed without the obligatory parasites.
5. **Shortening of breeding cycle:** Embryo condition is the primary factor for seed germination. In some plants, the embryo in seeds are present in rudimentary condition which requires a period of rest to form fully mature embryo. Under proper condition three years are required by the seed of *I. opaca* to complete their embryonic development and start germination and other plants are also on record for the same. Thus, *in vitro* culture of the embryo raises the embryo into an adult plant.

9.6 SUMMARY

In the development of plant biotechnology, we are witnessing a most dramatic evolution of new techniques applicable to Agriculture and Industry. One such Biotechnological application is Plant tissue culture which includes cloning of plants, large scale *in-vitro* propagation of plants of uniform or differing quality. The science of plant tissue culture comes with the work of Haberlandt (1902). He himself was unsuccessful but subsequent efforts by his successors were also dogged by failures. A fascinating outcome of tissue culture studies initiated at the University of Delhi has been the spectacular demonstration for the first time of the development of pollen embryoids and plantlets from anther culture of *Datura* by Guha and Maheshwari. Their work stumbled into the discovery which revolutionized plant breeding programmes of the future world.

Anther culture is a technique of culturing anther/pollen under aseptic *in vitro* condition to raise haploid plantlets. The purpose of anther and pollen culture is the production of haploid plants and the significance of haploids has been realized for a long time. Plant breeders are interested in haploid plants because either spontaneous doubling or an application of the chemical colchicines to double the chromosome number gives rise to homozygous plants. In the course of development of haploid plants certain factors such as Physiological status of the donor plants, Pre-treatment of cultured anthers, Culture medium, gaseous environment in culture tubes and light have their role in haploid plant production.

Embryo culture represents the earliest technique to obtain viable offsprings by culturing a young embryo from seed in a suitable culture medium to regenerate a complete plantlet. Embryo rescue literally mean to rescue an embryo and regenerate the embryo into viable plant *in vitro* using plant tissue culture technique which otherwise going to be aborted with the time due to several physiological or biological reasons.

Practically these tissue culture techniques have their potential role in producing homozygous line, overcoming seed dormancy, prevention of embryo abortion in early ripening fruits, regeneration of hybrid plants , seed germination, shortening of breeding cycle.

In conclusion, *in vitro* haploid production, embryo culture and embryo rescue, are some novel approaches to crop improvement. Despite of several problems this technology holds promises of newer achievements.

9.9 GLOSSARY

Artificial Seeds: A gel bed containing somatic embryo, shoot bud, necessary nutrient and growth regulators etc, needed for the development of complete plantlet.
Auxins: A class of growth regulators, that are primarily associated with enhancing cell division, cell elongation and root initiations in cultured cells. IAA, IBA, NAA and 2,4-D are some commonly used auxins in Plant tissue cultures.
Anther culture: Production of haploid plant by culturing anther on suitable culture medium.
Caulogenesis: Shoot regeneration from the cultured cells or stem organogenesis.
Colchicine: An alkaloid from the bulb of *Colchicum autumnale* that interferes with the formation of spindle apparatus.
Contaminations: Presence of unwanted cells or micro-organism in a pure or aseptic culture.
Dedifferentiation: Conversion of differentiated plant cells or from specialized organ into meristematic ones.
Differentiations: Development of different organs from the meristematic cells.
Haploid plants: Plants having half chromosome number of the species.
Hybrid rescue: Culture of embryo to save hybrid that otherwise die due to degeneration of endosperm.
Rhizogenesis: Root regeneration from the cultured cells in plant tissue culture.

9.8 SELF ASSESSMENT QUESTIONS

9.8.1. Short answer type questions

Q.1. What is androgenesis?

Q.2. What is shoot differentiation?
Q.3. explain cellular totipotency.
Q.4. Define Embryo rescue.
Q.5. What is surface sterilization and its importance in establishing culture.

9.8.2-Multiple choice questions:

1. Totipotency was first observed by

a. Steward b. Guha and Maheshwari
c. Strasburger d. Haberlandt

2. The basal medium comprises of:

a. Inorganic salt+ Vitamins+Sucrose b. Salts of major and minor elements only
c. Inorganic salts + growth hormones d. Inorganic salts + Sucrose + IAA+ Kn

3. Haploid plants are obtained by

a. Root tip culture b. Seed culture
c. Anthe culture d. Shoot tip culture

4. Androgenic haploid were first produced by

a. Guha and Maheshwari b. Maheshwari and Johri
c. Maheshwari and White d. Rangaswamy

5. Embryo culture was first tried in the members of the family

a. Cruciferae b. Leguminosae
c. Asteraceae d. Gramineae

6. The experimental plant piece subjected to tissue culture is referred to as

a. *In vitro* culture b. Explant
c. Nurse tissue d. Callus

Answer key 1. (d); 2. (a); 3 (c); 4. (a); 5. (a); 6. (b).

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9.11 TERMINAL QUESTIONS

Q.1. Name the common culture media used for various cultures. Discuss briefly their composition.

Q.2. Describe the basic aspects of plant tissue culture.

Q.3 Define anther and pollen cultures. Briefly describe the early patterns of cleavage in cultured pollen grains and the different modes of subsequent development of proembryogenic cell mass so obtained.

Q.4. Briefly describes the mode of production of haploid plants and their various applications for crop improvement.

Q.5. Briefly describe the technique of embryo culture.

UNIT-10: EXPERIMENTAL EMBRYOLOGY, POLLEN STORAGE AND TEST TUBE FERTILIZATION

10.1- Objectives

10.1 OBJECTIVES

After reading this unit, students will be able:

- To understand the definition of Embryology
- To understand the applied aspect of embryology.
- To understand different types of culture techniques.
- To understand different methods of storage of pollen grains.
- To understand test tube fertilization.

10.2 INTRODUCTION

In exact terms, embryology is defined as the branch of biology which deals with the study of events that lead to the formation of an embryo which represents future tiny plant present inside the seed or the study of proceedings of life cycle of a plant is referred as embryology. Embryology includes series of events *i.e.* study of carpel, stamen, formation of microspore and megaspore, development of male and female gametophyte, pollination, fertilization, formation of embryo and endosperm. We can say that the study of all the aspects of embryo is embryology and we feel proud by knowing that Professor P. Maheshwari is known as Father of Indian Embryology.

In angiosperms, the core plant body is sporophytic which is separated into root, stem and leaves. During reproductive phase, flowers are generated which are the most important parts concerned with sexual reproduction. Flower is believed to be a modified shoot with extremely reduced internodes and leaves are specified variously to act as diverse floral parts *i.e.* sepals, petals, stamens, and carpels which we have studied in earlier chapters.

10.3 EXPERIMENTAL EMBRYOLOGY

Experimental embryology or applied embryology is a division of embryology that deals with the mechanisms regulating the progressive development in plants by way of conducting experiments. It characterizes the phases of the determination of the material of fundamental structures and the portion of macromolecules in the progression of determination and isolation and the features responsible for morphogenesis.

The development in the discipline of experimental embryology has been feasible through the technique of plant tissue culture in which isolated plant cells or tissues or organs are grown in nutrient medium (either in glass or plastic containers) under accurate aseptic state. There are three important aspects of the plant tissue culture technique:

1. Nutrient medium

2. Upholding of strict aseptic state

3. Aeration of sophisticated tissue

Different cells and organs have its distinct nutritive requisite for finest growth. Most of the plant tissue culture media contain inorganic salts of macronutrients and micronutrients, vitamins and sucrose as the source of carbon. A media comprising of these ingredients referred as basal medium which also involves growth regulators (Auxin, cytokinin and gibberelic acid) either solitary or in several combinations. Natural plant extracts, casein hydrosylate, yeast extract, coconut milk and fruit juice are also added to the basal medium some time as per the specificity of the plant and this kind of medium is referred as supplemented medium.

Tissue culture is fundamentally centered on the concept of totipotency. This term is made up of two words *i.e*. toti= total and potency = potential which means the ability of a plant cell to grow into a complete plantlet (Fig.10.1.). The concept of totipotency was put forward by Haberlandt in 1902 and its practical facet was signified by Steward in 1932.

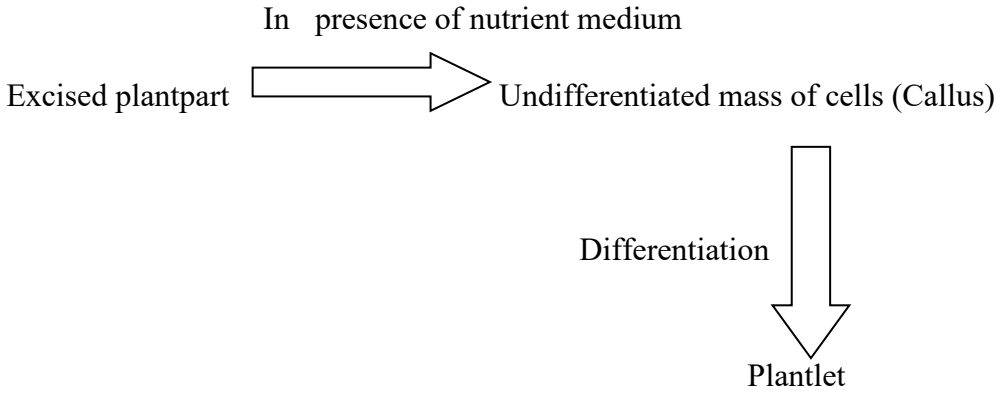

Fig.10.1. Representation of tissue culture technique

Different types of tissue culture techniques are as follows:

10.3.1 Embryo culture

Though we have discussed about the embryo culture in the previous chapter but to refresh the memory it is the sterilized segregation and development of an immature or mature embryo *in vitro* with the aim of gaining a practicable plant. The first effort to raise embryos

of angiosperms was formulated by Hanning in 1904. It is a vital technique in the propagation of plant species specifically for those whose propagation is impracticable by standard techniques or the plants yield seeds with low sustainability. It is of two types: **Mature embryo culture** and **immature embryo culture**.

It is a valuable contrivance to plant breeders as it results in the production of haploids, disabling dormancy of seeds, shortening of breeding cycle, to incapacitate self-sterility of seeds and production of healthy plants from non-viable embryos.

10.3.2 Endosperm culture

Endosperm is an outcome of double fertilization but unlike embryo, it progresses into an amorphous tissue. Endosperm culture is the *in vitro* expansion of isolated mature or immature endosperm from seed at an appropriate period on a proper culture medium to obtain plantlets which are triploid.

Triploids are usually self-sterile or linked with seedlessness, so these may be utilized for plants where seeds are not commercial product.

10.3.3 Protoplast culture

It refers to the culture of protoplast in either liquid or semisolid agar medium. Protoplasts are the cells devoid of cell wall isolated from the parenchymatous tissue by means of enzymatic procedures and then the feasible protoplasts are sterilized and refined.

The protoplast culture is intended mainly to raise genetically transformed plants and besides this the most significant feature of the protoplast culture is the creation of somatic hybrids that was first reported by Carlson in 1972 in *Nicotiana langsdorfii* × *Nicotiana glauca.*

10.3.4 Pollen culture

It is a technique where isolated pollen grains are cultured on a medium. A significant feature of plant tissue culture technique is the creation of haploids by pollen culture containing a single set of chromosome which is of vast importance in the initiation of mutation. First successful establishment of pollen culture was performed by Guha and Maheshwari in 1964 in *Datura inoxia.* Procedure involving production of haploids from pollens is also referred as **androgenesis** which was first observed by Tuckele in 1958 in *Gingko biloba*.

10.3.5 Cell suspension culture

Raising of individual cells (separated from explant) in liquid culture medium followed by placing in gyratory shaker to provide exposure to air and spreading cells is referred as cell suspension culture or it is a type of culture where distinct cell or small mass of cells

reproduce while deferred in stirred liquid medium. To attain perfect cell suspension, a friable callus is shifted to stirred liquid medium where it disrupts and freely diffuses. After eradicating the large callus portion, only distinct cell or small cell mass are again shifted to fresh medium and after two-three weeks, a suspension of vigorously growing cells is generated.

This suspension can then be proliferated by steady sub culturing into fresh medium. This technique is of great significance as the suspension culture originated from medicinally significant plants can be considered for the production of bioactive compounds *i.e.* secondary metabolites *viz.* alkaloids which play vital role in several pharmaceutical applications directing to an improved commercial value in recent years.

10.3.6 Callus culture

Callus is created by the multiplication of the parent tissue. The cells of callus are parenchymatous, living, amorphous and disorganized. Usually callus is formed as an outcome of wound at the split end of a stem or a root. When this tissue on culture generates disorganized mass of cells with no steady form then it is called as callus culture. The callus culture is not identical mass of cells because it is made up of two types of tissue *i.e.* differentiated and non- differentiated. Callus formation from an explant occurs in three successive phases: Initiation stage, cell division stage and cell differentiation phase

The complete plantlet can be rejuvenated in large number from callus culture through the manipulation of the nutrient and hormonal ingredients in the culture medium. This event is referred as **organogenesis** or **morphogenesis**. Additionally, callus tissue also serves as a good basis of genetic changeability as it is feasible to rejuvenate a plant from genetically alterable cells of the callus tissue.

10.3.7 Meristem tip culture

It refers to the cutting out of the tip of a shoot from a chosen donor plant for successive *in vitro* culture. Meristem tip culture technique was developed by Morel and Martin in 1952 to obtain virus free plants, disease resistant plants as well as for clonal propagation and proliferation.

10.3.8 Nucellus culture

This culture has been exploited to investigate the causes accountable for the development of adventive embryos. Adventive embryos are the embryos which ascend adventitiously from the cells of nucellus or integuments (*i.e.* Citrus, Mango). These embryos are of significant value to the horticulturist in the way that they are inherently identical and mimic the characters of maternal parent without inheriting the deviation carried by gametic fusion.

10.3.9 Seed culture

It refers to the *in vitro* culture of seeds in a culture medium to produce seedlings or plants. Seed culture technique is the best method for nurturing the disinfected seedling.

10.4 POLLEN STORAGE

In a nut shell, pollen is a product of chromosomal recombination and offers a consistent basis of genetic variety. It is a fine to rough dusty constituent encompassing pollen grains which are also referred as microspores are the male reproductive bodies of a flower. Each microspore is haploid, unicellular, and oval or rounded structures comprising of two layered wall. Outer layer is profused, cuticularized and rough called as exine which is mainly composed of a material called as sporopollenin which is extremely resilient to biological and physical disintegration. Inner layer which is called as intine is slightly thin and smooth and comprised of pectocellulose.

Different methods employed for the storage of pollens are as follows:

10.4.1 By depleting organic solvents

Various solvents viz. acetone, benzene, ethanol, chloroform, and phenols are largely considered as lethal to organism though it has been established that the pollen grains retained in these organic solvents can propagate *in vitro* and even influence the process of fertilization.

10.4.2. Cryogenic storage

This method of pollen storage involves preservation of pollens in liquid nitrogen at -196° C so that the pollens remain practicable for extended duration as at this super low temperature, pollens go through insignificant metabolic fluctuation in terms of physiological and biological processes.

10.4.3 Dry and cold storage

Several attempts have been made by embryologists to expand the viability of pollens by the regulation of temperature as well as relative humidity. Sub-freezing temperature *i.e.* from -5° C to -10° C and low relative humidity ranging from 25 to 50 % generally sustained optimal for keeping pollens in practicable state.

For directing pollens from one place to another, the preservation of pollens under regulating conditions involving both temperature and relative humidity is exclusive and undesirable. Thus King in 1959 originated a new method *i.e.* freeze frying which comprises the removal of water from the pollens after icing followed by sealing in vacuum or inert gas. There are several advantages of pollen storage:

1. To confirm the accessibility of pollens throughout the year without depleting synthetic growth chamber.
2. To deliver material for global exchange of germplasm.
3. Storage of germplasm for extended period.
4. To offer a continuous stock of short lived pollens.
5. To simplify additional pollination for refining yield.

10.5 TEST TUBE FERTILIZATION

A milestone technique established newly in the field of plant biotechnology is test tube fertilization which is defined as the effective fusion of male and female gametes separated from higher plants under *in vitro* state followed by successive restoration of fusion product into embryo which finally terminate into a plant.

Test tube fertilization occurs in three different ways which are as follows:

10.5.1 Stigma fertilization

In this method, emasculated flowers are first disinfected followed by isolation under *in vitro* condition. This fertilization has gained success in various plant species *viz. Nicotiana rustica*, *Glycine max*, *Pisum sativum* etc.

10.5.2 Placental fertilization

In this procedure, first complete flower is disinfected and placenta explant with unfertilized ovule is separated followed by inoculation into a nutrient medium. At the same time, anthers which are inaccessible and would be just on the verge of opening under *in vivo* conditions are sterilized. The anthers are released under disinfected state and pollen grains are retained near the ovules. After this process, certain period is needed to regulate whether pollen grains germinate, if they pass through the embryo sac followed by fertilization.

10.5.3 Fertilization of segregated ovule with placenta

This procedure of fertilization is somewhat similar to placental fertilization, here ovule is segregated *in vitro*, however, this method acquired slight achievement as it is really challenging to induce formation of embryo in *in vitro* fertilized ovules,

However, test tube fertilization requires some specific conditions in terms of selection of nutrient media that play vital role to encourage growth of embryo. Temperature may also be a pivotal factor and when the germ free flowers are used in the procedure of

stigma fertilization, proper attention should be taken that the stigma is not in connection with the sterilizing agent for longer period otherwise the exudates on the stigma will liquefy.

10.6 SUMMARY

1. Study of proceedings of life cycle with reference to embryo formation of a plant is referred as plant embryology
2. Experimental embryology or applied embryology is a division of embryology that deals with the mechanisms regulating the progressive development of embryo in plants by way of conducting experiments.
3. Tissue culture is fundamentally centered on the concept of totipotency.
4. Embryo culture refers to the *in vitro* preservation and culturing of mature and immature embryos in culture media.
5. Embryo culture is also referred as embryo rescue.
6. Endosperm is an outcome of double fertilization but unlike embryo it progresses into an amorphous tissue.
7. Endosperm culture is the *in vitro* culture of isolated mature or immature endosperm from seed at an appropriate period on a proper culture medium to obtain plantlets.
8. Protoplast culture refers to the culture of protoplast in either liquid or semisolid agar medium.
9. The culture where distinct cell or small mass of cells reproduce while deferred in stirred liquid medium is known as cell suspension culture.
10. Meristem tip culture is the culture of shoot or root tips containing meristematic tissue in a nutrient medium and it is an important strategy to obtain virus free plants, disease resistant plants as well as for clonal proliferation.
11. Adventive embryos are the outcome of nucellus culture.
12. Pollen storage is beneficial for genetic preservation, self-incompatibility, artificial pollination and for breeding programme.
13. Durability of pollen fluctuates greatly with plant species and storage surroundings.
14. Pollen storage involves different methods *i.e.* cryogenic, cold and dry storage as well as preservation in organic solvents.
15. Pollen storage offers storage of germplasm for extended period.
16. Test tube fertilization is an effective fusion of male and female gametes separated from higher plants under *in vitro* state followed by successive restoration of fusion product into embryo which finally terminates into a plant.

10.7 GLOSSARY

Fertilization: It is the union of male and female gametes to produce zygote.

Flower: It is the reproductive structure of plants.

Embryology: It is the branch of biology that deals with the formation, structure, development, and functional activities of embryo.

Totioptency: It refers to the ability of every plant cell to grow into a complete plantlet.

Callus: It is referred as the undifferentiated mass of cells.

Protoplast: It is the entire content of a cell without cell wall.

Pollen: It refers to the microspore which is a fertilizing element of the flowering plants.

Cell: It refers to the structural and functional unit of life.

Meristem: It is the region of the plant that is in a state of active division.

Seed: It refers to an embryonic plant.

In vitro: It refers to the experiment or procedure that is performed in artificial environment *i.e.* within a test tube or glassware.

10.8 SELF-ASSESSMENT QUESTIONS

10.8.1 Multiple choice questions:

1. The concept of totipotency was given by-

(a) Haberlandt (b) Karl Eris

(c) Max Swis (d) None of the above

2. The first attempt to raise the embryo of angiosperms was performed by-

(a) Hanning (b) Haberlandt

(c) Karl (d) None of the above

3. What is the outcome of double fertilization?

(a) Endosperm (b) Zygot

(c) Both a and b (d) None of the above

4. The technique where isolated microspores are cultured on a nutrient medium referred as

(a) Pollen culture (b) Protoplast culture

(c) Seed culture (d) None of the above

5. First successful establishment of pollen culture was performed by-

(a) Hanning in 1902 (b) Guha and Maheshwari in 1964

(c) Both a and b (d) None of the above

6. Which of the following technique is best for production of bioactive compounds?

(a) Cell suspension culture (b) Callus culture

(c) Seed culture (d) None of the above

7. Callus is referred as
(a) Unorganised mass of cells (b) Liquid culture
(c) Solid culture (d) None of the above

8. Which of the following culture technique results in the production of virus free plants?
(a) Meristem tip culture (b) Callus culture
(c) Embryo culture (d) None of the above

9. *In vitro* culture of seeds in a culture medium to produce plantlets referred as-
(a) Seed culture (b) Embryo culture
(c) Callus culture (d) None of the above

10. Cryogenic storage refers to
(a) Storage in liquid nitrogen at -196° C (b) Storage in hydrogen
(c) Both a and b (d) None of the above

11. Example of Stigma fertilization is
(a) *Glycine max* (b) *Nicotiana rustica*
(c) *Pisum sativum* (d) All of the above

Answer key: 10.8.1. 1. (a); 2 (a); 3 (c); 4 (a); 5 (b); 6 (a); 7 (a); 8 (a); 9 (a); 10 (a); 11 (d).

10.8.2. Fill in the blanks:

1. Fusion of male and female gametes under *in vitro* condition is ……
2. Adventive embryos are those ……
3. …….is a fertile portion where pollens are produced.
4. The cells of callus are ……. in nature.
5. Meristem tip culture was developed by……
6. Callus tissue serves as a good basis of …….
7. Adventive embryos occur in…….
8. Outer layer of pollen comprised of……..
9. Inner layer of pollen comprised of……..
10. Experimental embryology is also called as…….

Answer key: 10.8.2: 1. Test tube fertilization
2. Which arise adventitiously from the cells of nucellus or integuments
3. Anther
4. Parenchymatous

5. Morel and Martin in 1952
6. Genetic changeability
7. Citrus, Mango
8. Sporopollenin
9. Pectocellulose
10. Applied embryology

10.8.3. True and False

1. Endosperm culture is *in vivo* expansion of mature or immature endosperm.
2. Protoplasts are cell containing cell wall.
3. Production of haploids from pollens is referred as androgenesis.
4. Cells are structural and fundamental unit of life.
5. The cells of callus are parenchymatous.

Answer key: 10.8.3: 1. False**,** 2. False**,** 3. True**,** 4. True**,** 5. True

10.8.4 Very short answer type questions

1. What are different methods of Pollen storage?
2. Define experimental embryology?
3. What are the important aspects of plant tissue culture?
4. Define basal medium?
5. Define totipotency?

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10.10 TERMINAL QUESTIONS

10.10.1 Short answer type questions:

1. Describe the process of cryogenic storage.
2. What is placental fertilization.
3. Discuss stigma fertilization.
4. Describe a flower in detail.
5. What are the differences between dry and cold storage and their applicability.

10.10.2 Long answer type questions:

1. Describe the reproductive phase of plant in detail.
2. Describe in detail different types of culture techniques.
3. Describe in detail experimental embryology.
4. Describe the advantages of experimental embryology.

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