BOTANY THEORY NOTES FOR

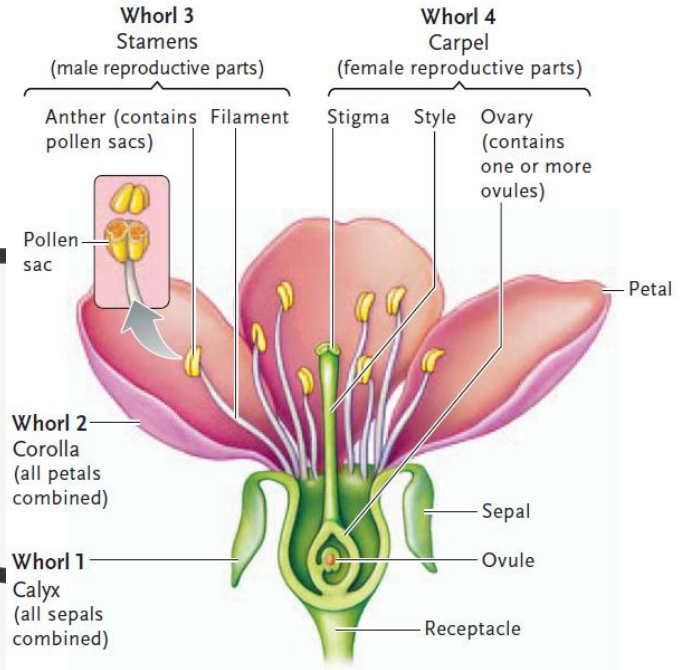
II BSc.

FOURTH SEMESTER

PAPER IV

GYMNOSPERMS AND EMBRYOLOGY OF ANGIOSPERMS

STRUCTURE OF A TYPICAL ANGIOSPERM FLOWER



Flower is the **reproductive organ unique** to **angiosperm** plants. It contains **male** and **female** reproductive organs, which are responsible for the production of gametes (sex cells). In general, a flower consists of four whorls, **calyx**, **corolla**, **androecium** and **gynoecium**. **Calyx** and **corolla** are called **accessory** whorls, while other two are reproductive whorls. **Androecium** is called **male** whorl, as it contains stamens, male sex organs. **Gynoecium** is called **female** whorl as it contains pistils (or carpels), the female sex organs.

Pedicel: Pedicel is the **stalk** of the **flower** which may be short, long or even absent. The pedicel and receptacle have typical structure, with a normal vascular cylinder. The cylinder may be unbroken or it may contain a ring of vascular bundles. In the region where floral organs are borne, the pedicel expands into the receptacle. The vascular cylinder also expands and the vascular bundles increase somewhat in number, and finally traces begin to diverge. In the simplest cases vascular traces for different organs and whorls of organs arise quite independently.

Floral whorls:

(a) Calyx: It is the **first** or **outermost protective** whorl. Individual member of calyx is called a **sepal** which is generally green. The sepals resemble leaves in their anatomy. Each sepal consists of ground parenchyma a branched system and epidermis. The chloroplasts are found in the green sepals but usually there is no differentiation into palisade and spongy parenchyma. The epidermis of sepals may possess stomata and trichomes.

(b) Corolla: It is the **second** or **attractive** whorl present inner to calyx. Each member of corolla is called a **petal**. The petals also resemble leaves in their internal structure. They contain ground parenchyma, a more or less branched vascular system, and an epidermis. They contain pigments-containing **chromoplasts**. Very often, the epidermal cells of the petals contain volatile oils which emit the characteristic fragrance of the flowers.

(c) Androecium: It is the **third** or **male** whorl. It is a collection of male parts called **stamens**. Each stamen is a modified leaf or microsporophyll. Each stamen consists of **3 parts** – **filament**, **anther** and **connective**. Each anther has two anther lobes and each lobe usually contains two pollen sacs or micro-sporangia filled with pollen grains or microspores.

(d) Gynoecium or Pistil: It is the **fourth** or **female** whorl, arid its functional units are called **carpels** (= megasporophylls). A typical carpel consists of **ovary**, **style** and **stigma**. Ovary is the swollen basal part of the carpel that contains one or more ovules. Each **ovule connected** to the **ovary** wall through a special tissue called **placenta**.

ANDROECIUM

The androecium is the floral organs composed of stamens or micro- sporophylls. Collectively, all the stamens are referred to as the **androecium.**

Stamens or Micro-Sporophylls

Each stamen consists of 3 parts – filament, anther and connective.

Filament: Ordinarily, each stamen is composed of a slender **stalk**-like filament **supporting** a **knob-like spore case or the anther** which permits exsertion of pollen out of flower. It is **attached** to the **receptacle** or arise from the **petals** (epipetalous). The structure of filament is quite simple. The epidermis is cutinized and bears trichomes. The stomata may also be found in the epidermis of both anther and filament. The vascular bundle is found throughout the filament and culminates blindly in the connective tissue situated in between the two anther lobes.

Connective: Ordinarily, the connective is a patch of **sterile tissues** connecting the two parallel anther lobes .It is a prolongation of the filament and contains the **conducting strands**.

Anther: fertile portion of stamen that dehisces to release **pollen grains**; composed of **anther sacs.** Each anther has two anther lobes (**bilobed**) and each lobe usually contains two pollen sacs or micro-sporangia placed

longitudinally. There are longitudinal grooves or sutures along the ventral face of the anther demarcating the pollen chambers. Each pollen chamber represents a microsporangium and contains **innumerable fine, powdery mass called microspores or pollen grains**. The stamen, therefore, is a microsporophyll bearing four microsporangia (**quadrilocular** or **tetrasporangiate**).

MICROSPORANGIUM

Structure and Development of Microsporangium or Mature Anther

Young anthers are more or less **oblong** in shape in section and made up of homogeneous mass of **meristematic cells** without intercellular space (Fig. 3.1 A). With further development, the anther becomes 4-lobed. The microsporangia are developed inside the corners of the 4-lobed anther.

The **outer layer** of anther is called **epidermis**. Below the epidermis, at **each corner**, some cells become **differentiated** from others by their dense protoplasm and conspicuous nuclei — **archesporium** or **archesporial cells** (Fig. 3.1 B). Each **archesporial** cell then **divides** mitotically and forms an **outer primary parietal** cell and an **inner primary sporogenous** cell.

There may be only one such archesporial cell in each of the four lobes as in ***Boerhaavia***, etc., or there may be more of them forming a plate (***Ophiopogon***, etc.). Longitudinally, also, there may be one to many of them.

The outer primary parietal cells form primary parietal cell layer at each corner (Fig. 3.1 C). 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 and anticlinally and form **multilayered antheridial wall (3-5 wall layers, *i.e.*, endothecium, middle layers and tapetum)** (Fig. 3.1 D).

The **innermost layer** of antheridial wall, which remains in close contact with the sporogenous tissue, functions as **nutritive layer**, called **tapetum** (Fig. 3.1 D).

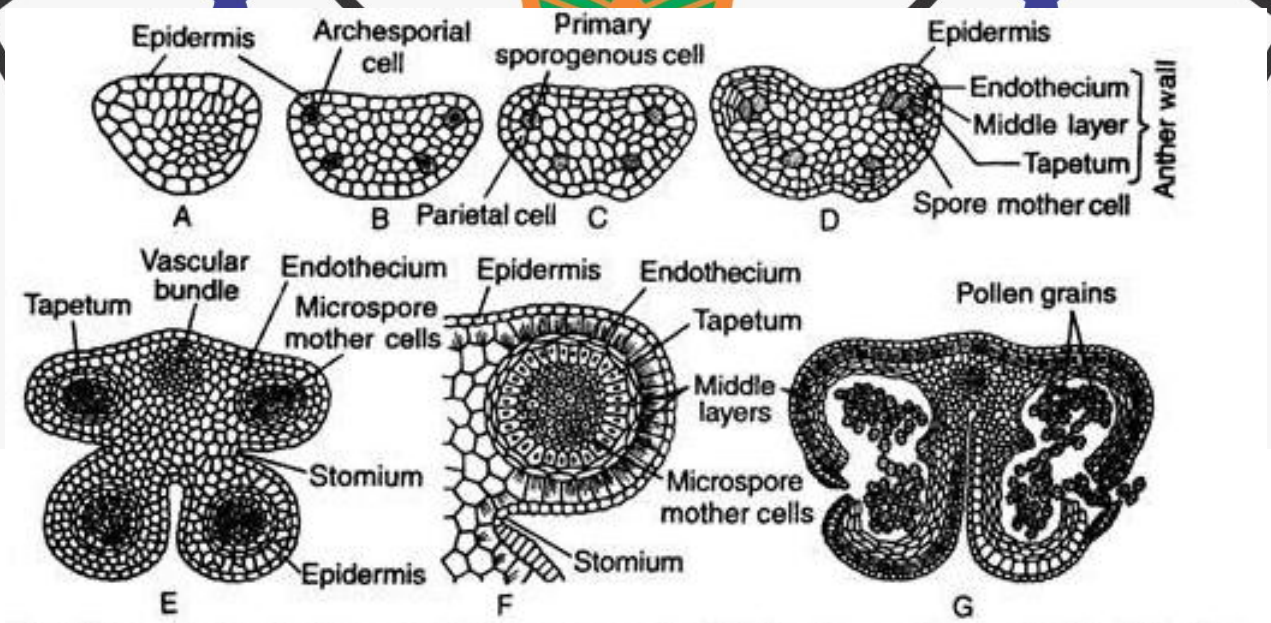
The **primary sporogenous cells** either **directly** function as **spore mother cells** or **divide mitotically** into a **number of cells** which **function** as **spore mother cells** (Fig. 3.1 D).

Between the tapetum and the endothecium there are one to **three middle layers of cells**. The middle layers and the tapetum are usually crushed by the time actual meiosis occurs in the sporogenous cells.

During **microsporogenesis** (i.e., development of microspores or pollens) the **nucleus** of each **microspore mother cell** undergoes **meiosis** or reduction division ultimately giving rise to **four haploid** (i.e., possessing 'n' number of chromosomes) **nuclei**. These four nuclei are arranged tetrahedrally and are soon invested with cell walls. They are now the **microspores** or **pollens** which soon **dry up** and become **powdery** while the **tapetum** becomes **absorbed**.

The anther now becomes a dry structure, the partition walls between the sporangia (i.e., loculi) are usually destroyed and the microspores (pollens) are soon liberated by dehiscence of the anther.

The **tapetal cells** often become **multinucleate** and play a great part in the **nutrition of the pollens**. Sometimes they develop a Plasmodium after disintegration and play a part in the development of the exine of the pollen. Even a part of the sporogenous tissue may break down and serve for nutrition instead of developing spores.



3.1 : Stages of anther development and microsporogenesis : A–D. Developmental stages, E. T.S. of developing anther, F. Enlarged microsporangia with wall, and G. T.S. of mature anther showing liberation of pollen grains

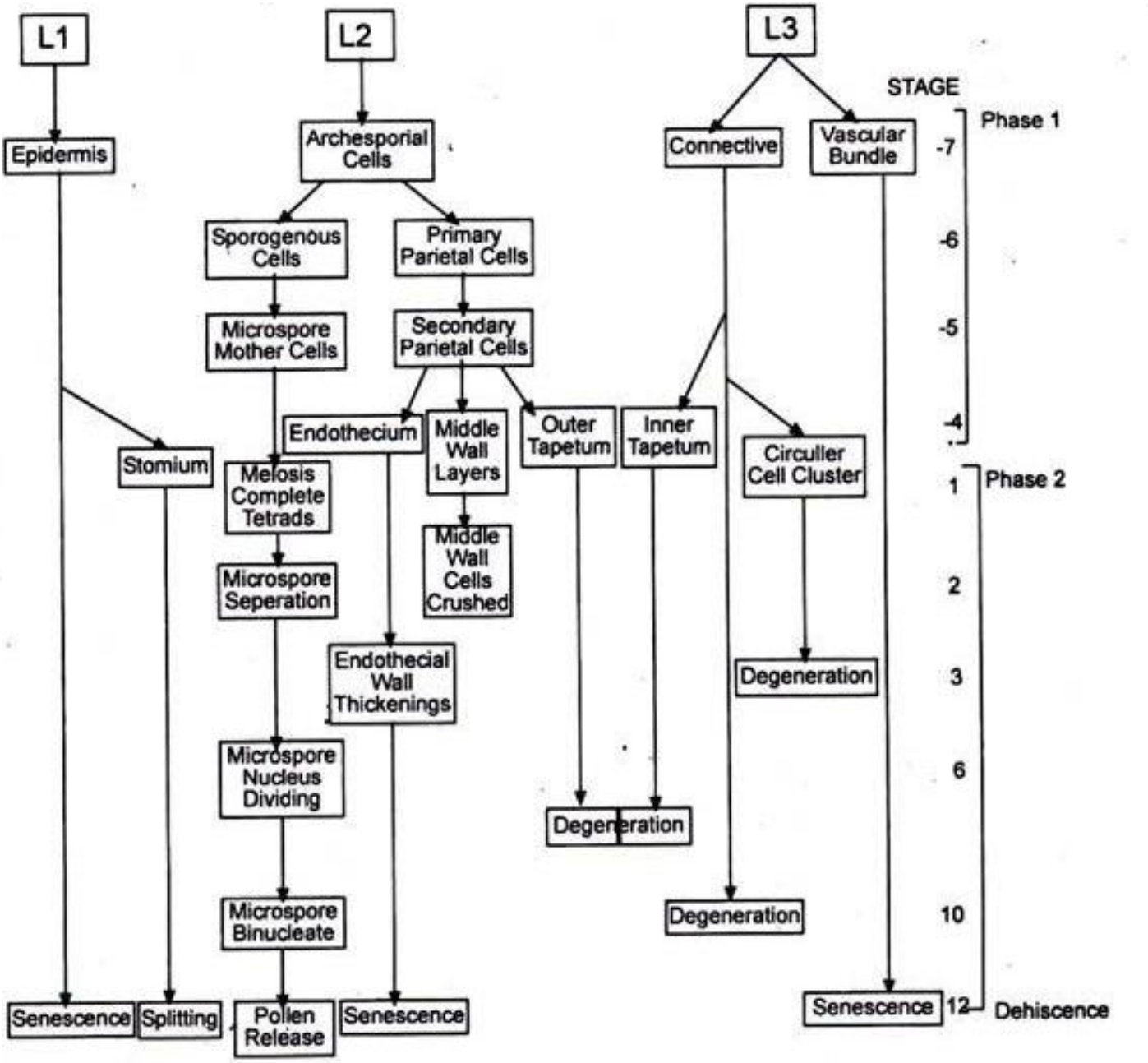


Fig. 1.2 : Cell lineage and major events that occur during anther differentiation and dehiscence along with histological observations of the L1, L2 and L3 derivatives of a tobacco stamen primordium.

ANTHER WALL LAYERS:

Epidermis, endothecium, middle wall layers (2-3 layered), and the tapetum (outer and inner layers) constitute the anther wall.

i. Epidermis:
The epidermis is an outermost, single layered protective sheath of the anther. It divides anticlinally and tries to keep space with the enlarging internal tissues of the anther. As a consequence, they undergo considerable stretching and flattened in surface area. It provides the structural integrity to the anther, assists in gaseous diffusion, prevents moisture loss, and in the dehiscence of the anther lobes. Sometimes the epidermal cells may be cutinized or lignified.

ii. Endothecium:
The outer most layers of the descendants of the parietal cell located immediately below the epidermis are called the endothecium. It attains the maximum development before the dehiscence of the anther. The cells are radially elongated and decorated with fibrous bands (hence this layer is called fibrous layer and are hygroscopic in nature) that run upward from the inner tangential walls, ending near the outer wall of each cell as an incomplete ring. The outer tangential walls remain thin. The endothecium is associated with high proportion of α-cellulose and small amount of lignin at maturity. The specialized nature of the endothecium together with the stomium helps in the dehiscence of the anther.

iii. Middle Layer:
Below the endothecium 2-3 layers of cells are present which constitute middle layers.
I middle layer: - This is developed from upper middle layer of outer secondary parietal layer.
II middle layer: - present below the I middle layer which also develops from upper middle layer of outer secondary parietal layer.
III middle layer: - present below the II middle layer which are derived from inner secondary parietal layer.
The cells of the middle layer are usually ephemeral and become flattened and crushed by early meiosis in the pollen mother cell. The layer adjacent to the endothecium may even develop fibrous thickenings. In few instances it also serves to store starch that is later mobilized to the developing pollen.

iv. Tapetum:
The tapetum is the innermost layer of the anther wall and is usually derived from the inner secondary parietal layer. The tapetum surrounds the sporogenous tissue and attains maximum development when the microspores are in the tetrad stage, after which they go into decline that results in the collapse of the cells. Usually tapetum consists of single layer of cells or it may contain 2 to 3 layers. As the tapetum completely surrounds the sporogenous tissue, major part of it is derived from parietal cells and a small part developed from the sporogenous tissue. Tapetum transports the nutrients to the developing sporocytes. Tapetal cells are pigmented and it is red brown in apple or violet in Anemone.

A. Characteristic of Tapetal Cells:
a. They are distinctly enlarged or radially elongated and always ephemeral.
b. The cytoplasm is dense with prominent nuclei. It contains ribosomes, mitochondria, Endoplasmic reticulum, many vesicles and active organelles.
c. Cells may be multinucleate or polyploid and are comparatively rich in DNA.
d. There is irregular mitotic divisions and nuclear fusion.
e. They are characterized by rapid and intense activity with degeneration of their cytoplasm.

B. Behaviour of the Nucleus in the Tapetal Cells:
Tapetal cells undergo dynamic fluxes during their short life span. The characteristic cytological feature of the tapetal cells, irrespective of the type, is the increase in the content of their DNA, which is initiated with meiosis in microsporocytes and extends through the meiotic division.
Since DNA increase is not followed by regular mitotic division it results in certain cytological abnormalities, like multinucleate cells, endomitosis, polyploid nuclei, polyteny and endoreduplication.
Based on the behaviour, two kinds of tapetum were recognised.

a) Amoeboid or Invasive or Periplasmodial tapetum:

The inner and radial walls of the tapetum break down due to the action of hydrolytic enzymes and their protoplast containing food material penetrates between the pollen mother cells and developing pollen grains or moves into the inner anther cavity. After intrusion, they fuse with each other and forms a mass of tapetal periplasmodium or shows amoeboid movement around the pollen mother cells and forms the peri-plasmodium mass. This tapetal plasmodium remains associated with the pollen grains till their maturity by providing nourishment. When the anther gets drying up, the tapetal periplasmodium gets dehydrated and coated over the surface of pollen grains, thereby helping in the formation of exine. Amoeboid tapetum is considered as the primitive type. It is found usually in hydrophytes Eg:- ***Alisma, Tradescantia, Typha, Saggitaria, Potamogeton*.**

b) Glandular or Secretory tapetum:

The cells of glandular tapetum remains intact throughout microspore development. They secrete their substances from their inner faces. Secretary tapetal cells are thin and possess almost all cell organelles like mitochondria, plastids, dictyosomes etc. some spherical structures called proubisch bodies are also present. Just before the pollen mother cells undergo meiosis, the walls of the tapetal cells become thick and there is considerable increase in the number of ribosomes and pro-ubisch bodies with the completion of pollen development. Proubish bodies pass into the anther locule from the tapetal cells and they are now called ubisch bodies and they coated over the pollen grains Eg:- Higher monocots and many dicots.

Functions of tapetum:

1. The nutrients are transported through tapetum to the sporogenous tissue i.e., Secretion of polysaccharides into the locules during the free microspore stage, which are absorbed by microspores.
2. Tapetum is involved in the synthesis of callase which release microspores in a tetrad by degrading callose wall.
3. The role of the tapetal cells in the secretion of sporopollenin precursor is non-ambiguous however, its role in the synthesis of sporopollenin is not clear.
4. Tapetum plays an important role in Formation of pollen wall during post- meiotic period and in the formation of exine.
5. Formation of pollenkitt (Lipids and carotenoids) and tryphine, which are deposited on the pollen surface and helps to bind pollen grains together, and for efficient insect pollination. It is a insect attractant & protect pollen from ultra violet.
6. Tapetal cells have other activities that result in formation of different structures. In Onagraceae tapetal cells play a role in formation of fine flexible threads, known as viscin threads. In Asteraceae the tapetum forms an acetolysis resistant membrane outside the sporogenous tissue. This membrane is known as the culture sac or peritapetal membrane.
7. Formation of Ubisch bodies.

Sporogenous Tissue (also called microsporocyte)

The cells of sporogenous tissue have dense cytoplasm, nucleus, nucleolus and few small vacuoles. They are polygonal in outline and attached close to each other within a locule. The primary sporogenous tissues, formed by archesporial cells either directly function as spore mother cells or divide mitotically into a number of cells which function as spore mother cells (or microspore mother cells) these are diploid cells. Even a part of the sporogenous tissue may break down and serve for nutrition instead of developing into spores.

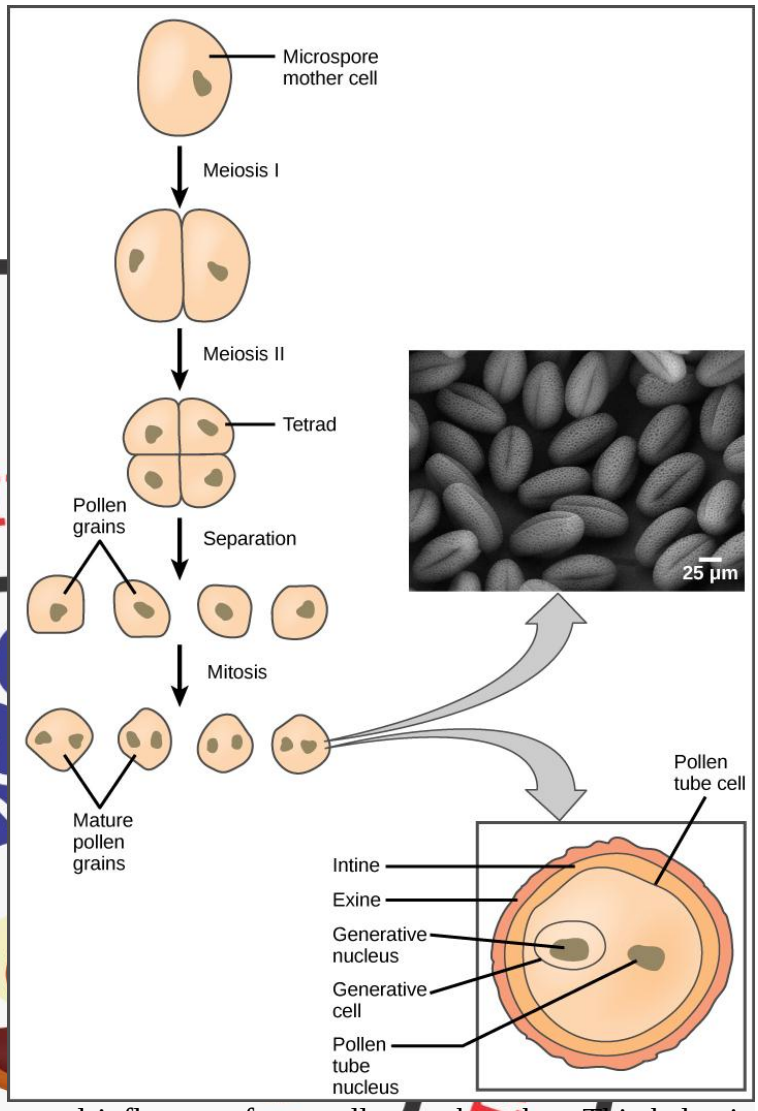
MICROSPOROGENESIS

The process of the formation and differentiation of microspores (pollen grains) from microspore mother cells (MMC) by reductional division is called microsporogenesis.

Pollen or Microspore mother cells (PMC or MMC)

The pollen mother cells are **polygonal in shape** and it is tightly packed until the anther begins to increase in size. All the PMC can form the **pollen grains** even then some of the cells **disintegrate** and acts as nourishment for the developing **pollen grains**.

The plasmodesmata interconnect the microsporocytes with one another and with the tapetum. Following the entry of the microsporocytes into the meiosis, the plasmodesmatal bridges with the tapetum is snapped. After the discontinuity of the plasmodesmata connection among the microsporocytes, it is covered by a primary wall made up of cellulose, and shortly before meiosis this wall disintegrates and is replaced by a massive deposit of callose (β-1,3- glucan), outside the plasma membrane. Callose deposition starts at the corners of the cells between the plasma membrane and the original wall. The deposition of the callose is initially incomplete, leaving many gaps through which there is an establishment of massive cytoplasmic channels between the microsporocytes.



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 the meiotic prophase the callose walls of the PMCs close up and the cytoplasmic channels are cutoff, with this all the PMCs become independent and go through the rest of the meiosis as isolated cells. Therefore, additional callose walls are formed between the daughter cells (microspore) of the PMCs. The enzyme callase disintegrates the callose (polysaccharide) present in pollen tetrad and the microspores are set free. While still within the callose wall the microspores start synthesizing their individual walls.

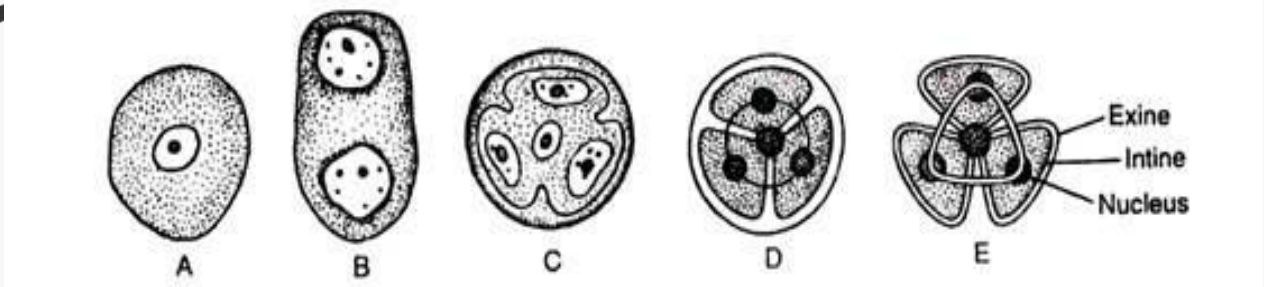


Fig. 3.2 : Different stages of development of microspore from microspore mother cell : A. Microspore mother cell, B. Diad stage, C. Tetrad stage, D. Clevage of protoplast and formation of pollen grains, and E. Four microspores i.e., pollen grains with exine and intine

Cytokinesis:

The microsporocytes after the meiotic division undergo cytokinesis, by any of the two processes, viz., simultaneous or successive.

i. Simultaneous Wall Formation:

In this case the **first meiotic division** is not accompanied by wall formation, as a result after meiosis I a **binucleate cell** is formed. The **two haploid nuclei** undergo the **second meiotic division** synchronously by virtue of their common cytoplasm. Later **callose walls** are laid down as **centripetally** growing furrows, which meet in the centre of the cell to produce a **tetrad**.

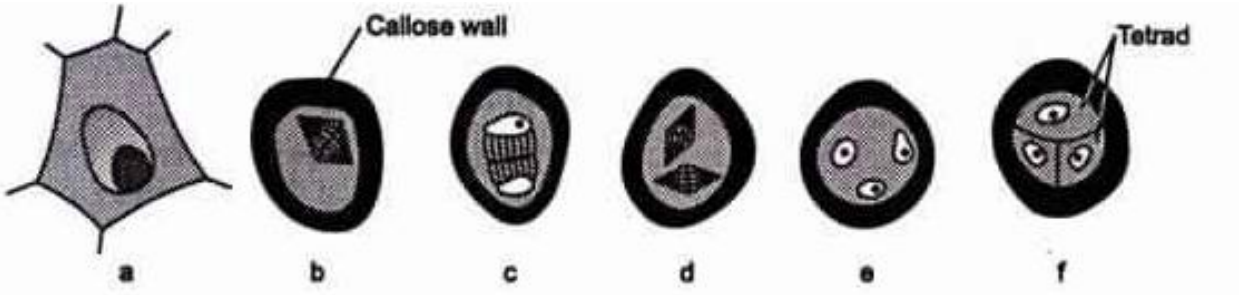


Fig.1.8 : Simultaneous type of cytokinesis in *Drimys winteri*. a-Pre-mitotic microspore mother cell without callose wall; b- Metaphase-I. c- Binucleate cell; d- Metaphase-II. e- Four nucleate stage. f- Tetrad stage.

ii. Successive Wall Formation:

Immediately after the **first meiotic division wall** is laid down **centrifugally** to form a **dyad**. The cell plate is formed in the center and then extends laterally. It is followed by the deposition of **callose** on either side of the plate. The cells of the dyad undergo **second meiotic division** followed by callose wall formation in the same as the first division, thus resulting in a **tetrad**.

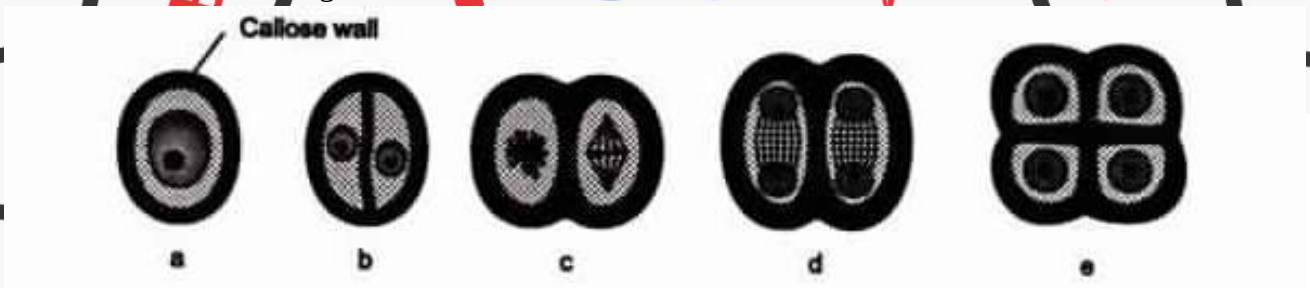
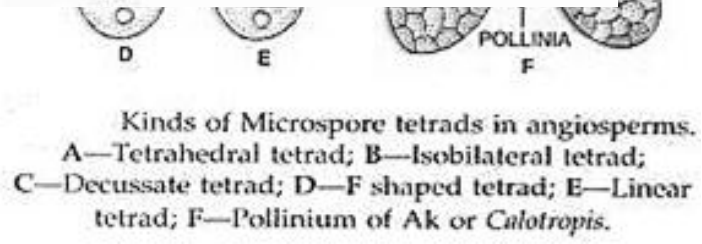


Fig.1.9 : Successive type of cytokinesis in *Commelina subulata* a- Pre-mitotic microspore mother cell; b- Dyad stage. c- Metaphase-II. d- Telophase-II. e- Tetrad stage.

Microspore Tetrad: The microspore so formed, remain associated with each other for some time. This group of 4n (pollen grains) is called microspore tetrad. The four microspores separate from each other, and each develops a characteristic shape or form which differs in different species of plants.



Kinds of Microspore tetrads in angiosperms. A—Tetrahedral tetrad; B—Isobilateral tetrad; C—Decussate tetrad; D—F shaped tetrad; E—Linear tetrad; F—Pollinium of Ak or *Calotropis*.

Different plants represent 5 types of tetrads –

1. **Tetrahedral tetrad (most common)**- Three spores in one plane and one in other plane. E.g., *Drosera*.
2. **Isobilateral tetrad**- All the four spores are formed in **one plane** because the spindles of first and second meiotic division remain at right angle to one another, e.g., *Zea mays*.
3. **Decussate tetrad**- Out of the **two lower spores**, only **one is visible**. Both the **upper ones are clear**; e.g., *Magnolia*.
4. **Inverted T shaped tetrad**- In meiosis II **upper cell** divides to form **two cells** present **side by side** and the **lower cell forms two cells** lying **one above the other**; e.g., *Aristolochia*.
5. **Linear tetrad**- All the four spores are present **one above the other** in a **linear** fashion; e.g., *Halophila*.
6. **Rhomboidal tetrad**- Spores are arranged in the form of rhomboid. E.g., *Anona muricata*.

Abnormalities-Pollinia, compound pollen grains.

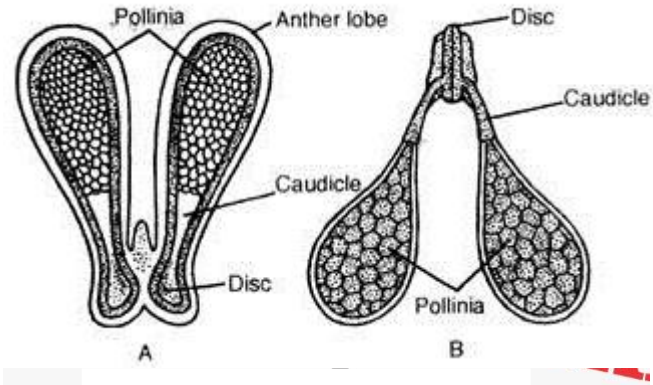
Occasionally there are either fewer than four spores resulting from the divisions of the microspore mother cell, or more than four. Less spore condition originates as the result of a failure of one division, or the formation of a "restitution nucleus" after the first division, or an irregular wall formation giving rise to one binucleate and two uninucleate spores. The formation of more than four spores (polyspory), usually results from the occurrence of lagging chromosomes which organize into micronuclei.

In general, however, such abnormalities in the number of microspores are found only in hybrids characterized by a high degree of sterility and the pollen grains arising in this way are nonfunctional. Usually the microspores soon separate from one another but in some plants, they adhere in tetrads to form the so-called **"compound" pollen grains** ex: - ***Anona, Drosera, Elodea, Typha*** and several members of the Apocynaceae, Asclepiadaceae, and Orchidaceae.

In the Mimosaceae there are larger units composed of 8 to 64 cells, and in a number of genera belonging to the **Asclepiadaceae** (***Calotropis procera***) all the microspores in a sporangium remain together to form a single mass called the **pollinium**. **Pollinium** occurs in pair forming balloon like structures. These structures are called pollinia. Each pollinium consists of a **stalk** (called **corpusculum**), **caudicle** (**disc** like) and two pollinia carries mass of pollen grains.

The family **Orchidaceae** is especially interesting in this connection. In some genera, such as *Cypripedium* and *Vanilla*, the microspores separate from one another and become free. In *Pogonia* the four cells of a tetrad adhere and form a compound pollen grain. In the tribes Ophrydeae and Neottieae this tendency is carried further and the compound grains are themselves held together in small units known as **massulae**. Finally, in *Coelogyne* and *Pholidota* all the microspore mother cells and their derivatives remain together and continue their development as a single unit.

Polyspory in *Hyphaene* is due to divisions of the members of the tetrads, Tetrads with as many as eleven microspores have been observed in *Cuscuta reflexa*. Such polyads have been reported in *Eupatorium odoratum* and *Thunbergia mysorensis*.



A. POLLINIA OF Orchid
B. *B.* POLLINIA OF *Calotropis*

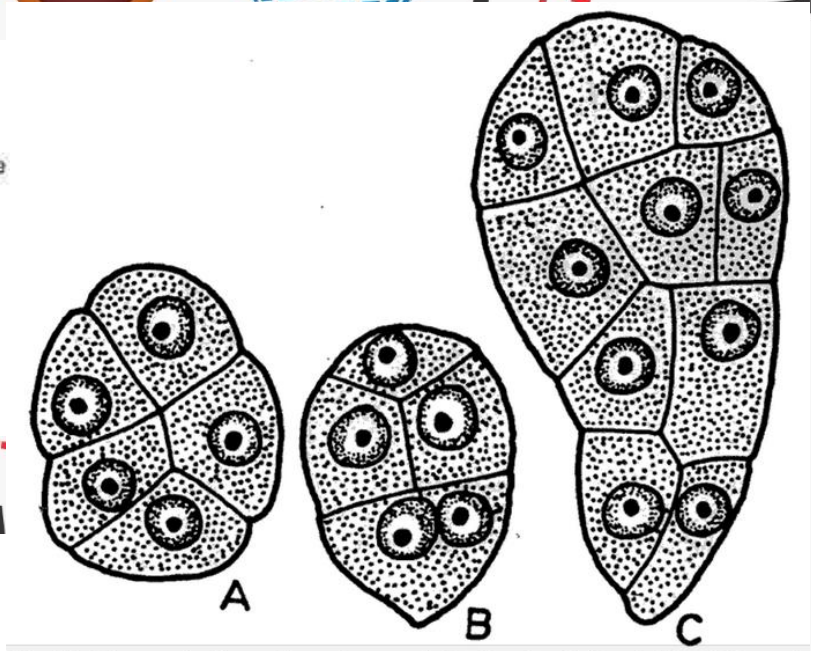


Fig. 2.7 Polyspory in *Eupatorium odoratum*. A, B. Pentads. C. Decad (after Maheswari Devi and Pullaiah, 1977).

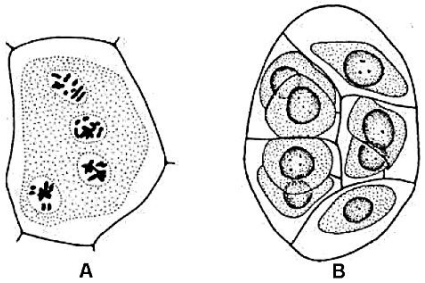


Fig. 3.15 Polyspory in *Hyphaene indica*. **A.** Microspore mother cell after meiosis. The four daughter nuclei are seen in division. **B.** Octad; very likely derived from the microspore mother cell shown in **A.** (*after* Mahabale and Chennaveeraiah, 1957)

DEVELOPMENT OF MALE GAMETOPHYTE (MICROGAMETOGENESIS):

Microspore or pollen grain is the first cell of male gametophyte (partially developed). The development of male gametophyte from pollen grain is called microgametogenesis.

During early stage of development, it remains within the microsporangium. The nucleus of the microspore begins to divide very soon after it is formed. Such division may begin even before the microspores dissociate from the tetrad condition.

The cell undergoes unequal division and forms a small generative cell and a large vegetative or tube cell (Fig. 3.5B). Initially the generative cell remains lying at one corner of the spore wall. A *callose layer* is deposited around the generative cell.

Generative cell wall appears in between plasma membrane of generative cell and intine towards the lower side and develops completely till it encircles the whole generative cell. Within short time, it gets detached from intine (The callose layer than dissolves) and becomes ellipsoid or fusiform or vermiform in shape (helps for penetration through pollen tube) (Fig. 3.5C) and remains suspended in the cytoplasm of the vegetative cell (2-celled stage i.e., vegetative cell and generative cell). Wall of generative cell disappears and its cytoplasm remains enclosed by Plasma membrane (its own and vegetative cell's).

Increase DNA synthesis initiates the second division i.e., the generative cell divides and gives rise to two non-motile ellipsoidal or lenticular or spherical cells — the male gametes (3-celled stage i.e., vegetative cell and two male gametes, Fig. 3.5D) which remain in close association.

This division may take place either in the pollen grain or after pollination or in the pollen tube which develops through germ pore after pollination or before reaching the embryo sac (more common) or rarely after entering embryo sac.

The nucleus of the vegetative cell is commonly known as tube nucleus (Fig. 3.5D). It usually shows sign of degeneration with the maturation of generative cell. Finally the tube nucleus remains within spore or may enter the pollen tube (Fig. 3.5E, F and G). Sooner or later it may be degenerated completely.

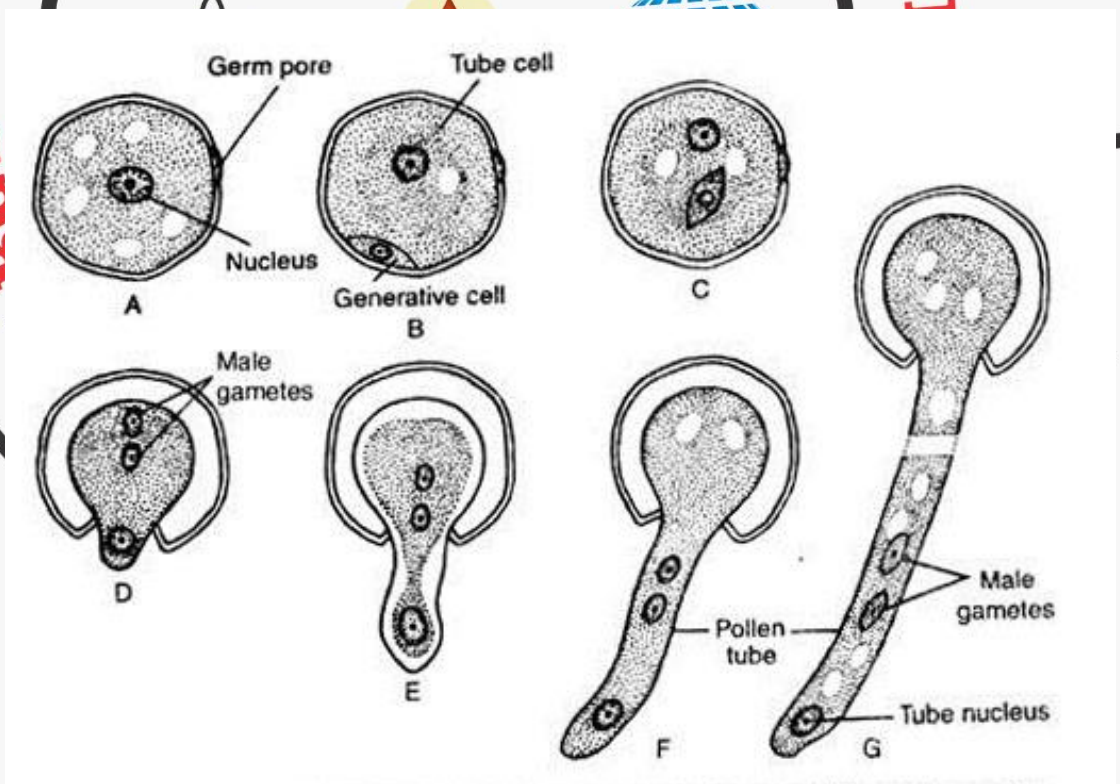


Fig. 3.5 : A-G. Germination of the pollen grain and development of the male gametes

OR

FORMATION OF VEGETATIVE AND GENERATIVE CELLS

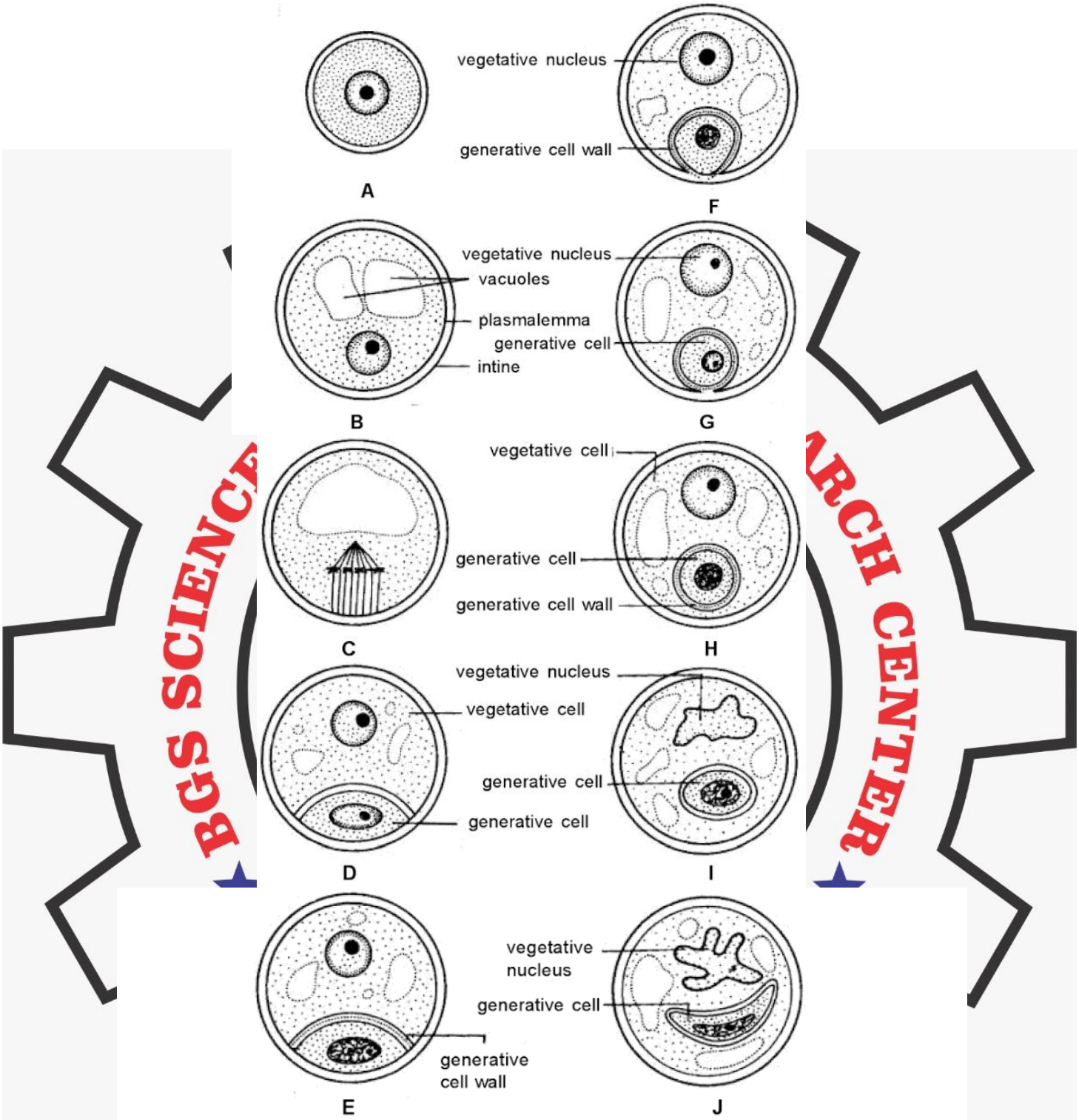


Fig. 4.1 Diagrammatized stages in the formation of vegetative and generative cells, generative cell wall formation and pinching-off of the generative cell into the cytoplasm of the vegetative cell. **A.** Microspore soon after release from the tetrad. **B.** The cytoplasm of the pollen grain has become highly vacuolated, and the nucleus has been displaced to one side. **C.** Pollen mitosis; note the asymmetric spindle. **D.** Two-celled pollen soon after pollen mitosis. **E.** Generative cell wall has appeared in between the plasma membranes of the vegetative cell and the generative cell. **F-H.** Stages in the detachment of the generative cell from the pollen wall. Immediately after detachment the generative cell appears spherical. **I.** The vegetative nucleus has become lobed and the generative cell has lost its spherical shape. **J.** The generative cell has become ellipsoidal.

Structure of male gametophyte (may be Microspores i.e., Pollen grains or pollen tube)

Microspore i.e., the pollen grain, which contains only one haploid nucleus. Microspore nucleus divides mitotically to form a smaller generative cell lying next to spore wall and a much larger vegetative cell (or tube cell). The pollen grains are shed from the anther at this bicelled stage (rarely three celled).

Vegetative cell continues to grow with increase in the number of cell organelles. Cytoplasm becomes dense, vacuole disappears along with increase in RNA and protein content. Nucleus highly convolutes and at maturity no nucleolus exist.

The generative cell is contained within the larger pollen tube cell. Upon germination, the tube cell forms the pollen tube through which the generative cell migrates to enter the ovary. Initially generative cell is spherical but later elongates to an extent of thread like to facilitate the movement into pollen tube. Cytoplasm contents are limited with normal cell organelles such as poorly developed mitochondria, ribosomes, endoplasmic reticulum, microtubules, dictyosomes and rarely plastids (if present, without starch). Generative cell undergoes mitotic division before or after the release of pollen to form sperms or male gametes (microgametogenesis).

Abnormalities – Nemec phenomenon (Pollen Embryo Sac)

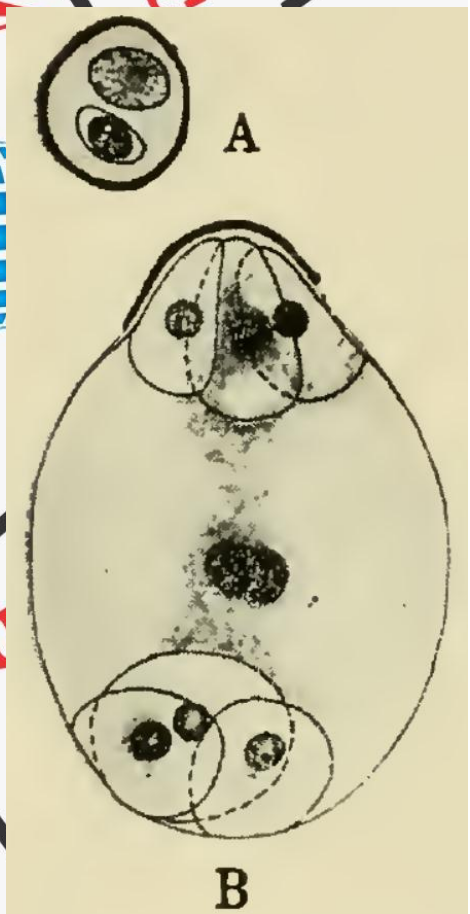
Pollen grains usually have two nuclei. Under normal conditions, dominance of male potency was observed in the petaloid. Here, a male gametophyte was usually two or three nucleated. Occasionally in some abnormal pollen grains the number of nuclei may increase (due to necrohormones), dominance of female potency was observed. In some members of Lilliaceae, however the number of nuclei increases to such an extent, that they resembles embryo sac. Such pollen embryo sacs were first reported in the petaloid anthers of ***Hyacinthus orientalis*** by **Botanist, Bohumil Nemec** in 1898. Occurrence of pollen embryo sac has also been reported in ***Ornithogalum nutans***.

Pollen embryo sacs are also called **NEMEC PHENOMENON**, as Nemec discovered them for the first time. According to Nemec, **pollen embryo sacs** are produced by the **repeated divisions of vegetative nucleus** while the generative nucleus degenerates.

Pollen embryo sacs have varying number of **nuclei ranging from 4-16**. They may or may not be organized. In addition certain abnormal pollen-embryo sacs were also seen, showing the following types of organization:

(1) 8 nuclei forming an egg, two polars, and five antipodal cells;
(2) 4 nuclei forming an egg, two polars, and one antipodal cell;
(3) 4 nuclei forming a polar and three antipodal cells but no egg;
(4) 16 nuclei forming a 5- to 10-celled egg apparatus, one or two polars, and a few antipodal cells; and
(5) more than 16 nuclei without any definite arrangement.

Stow has observed even the **fusion of polar nuclei** in **some pollen embryo sacs**. According to **Stow**, it is **not the divisions of the vegetative or Generative nucleus** which give rise to the pollen-embryo sacs but those of the **microspore nucleus** itself. Once the vegetative and generative cells have been differentiated, further development is quite normal and no pollen-embryo sacs are formed. He also reports the existence of large number of dead pollen in association with pollen embryo sacs. He believes that the **dead pollen secrete a necrohormone**, which **initiates abnormality** in the surviving pollen.



A. **NORMALPOLLEN GRAIN SHOWING VEGETATIVE AND GENERATIVE CELL.**
B. **POLLEN EMBRYO SAC**

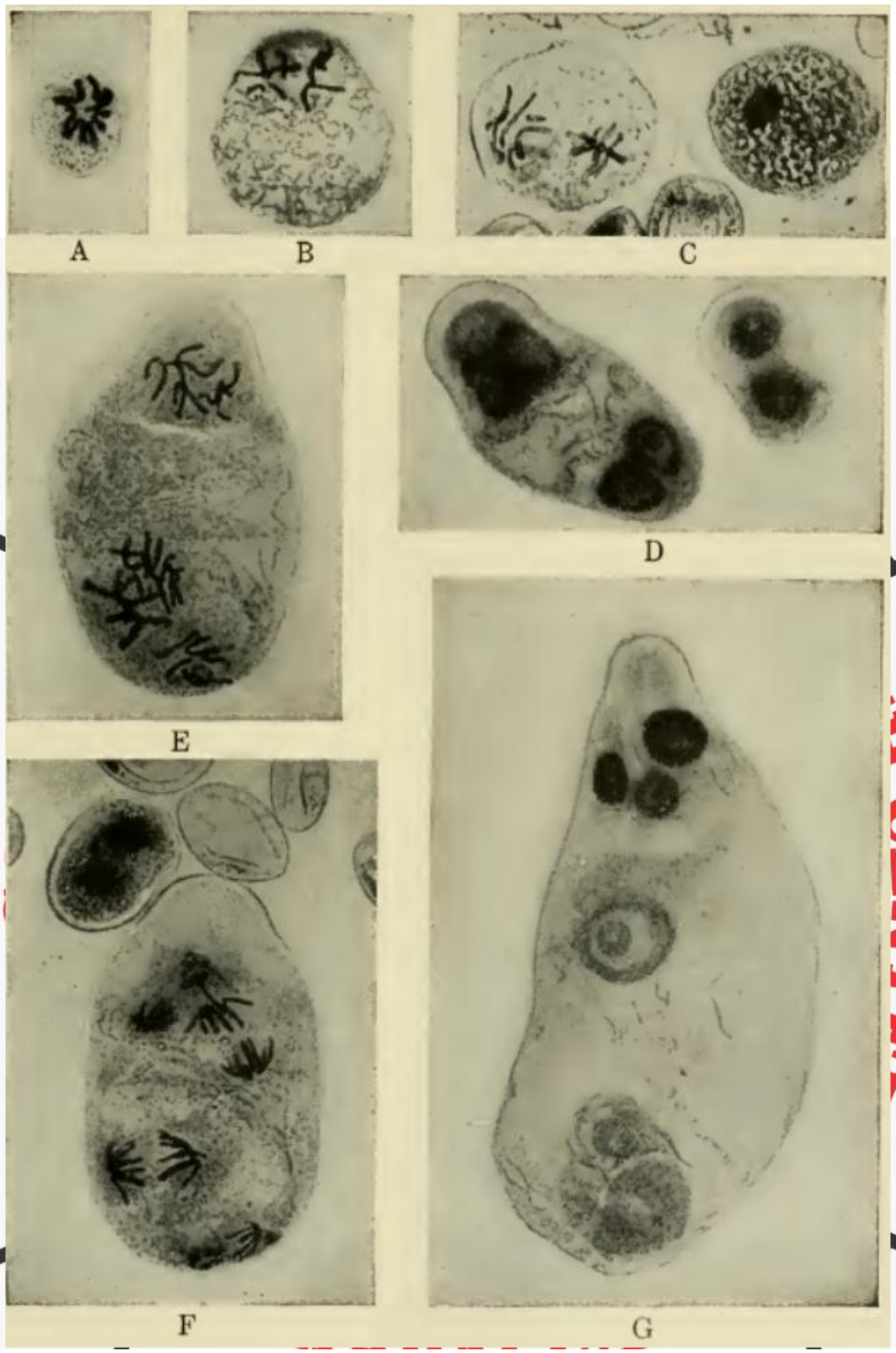
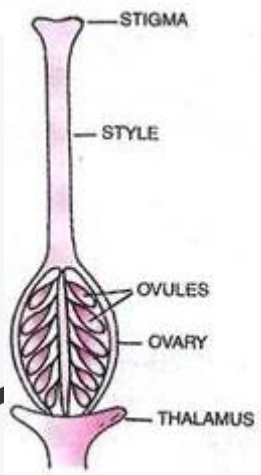


FIG. 105. Development of pollen-embryo sacs in *Hyacinthus orientalis*. *A*, microspore in metaphase of first division. *B*, microspore showing tendency toward formation of pollen-embryo sac; nucleus is in metaphase. *C*, second nuclear division in pollen-embryo sac; on right, young pollen-embryo sac with undivided nucleus. *D*, four- and two-nucleate pollen-embryo sacs. *E*, division of four nuclei; metaphase. *F*, same; anaphase. *G*, well-developed pollen-embryo sac.

STRUCTURE OF PISTIL

Gynoecium represents the female component of a flower. It may consist of only one carpel (monocarpellary), two carpels (bicarpellary), three carpels (tricarpellary) or many carpels (multicarpellary). Each carpel represents a megasporophyll. The free unit of gynoecium is called pistil. A pistil has three parts— stigma, style and ovary.



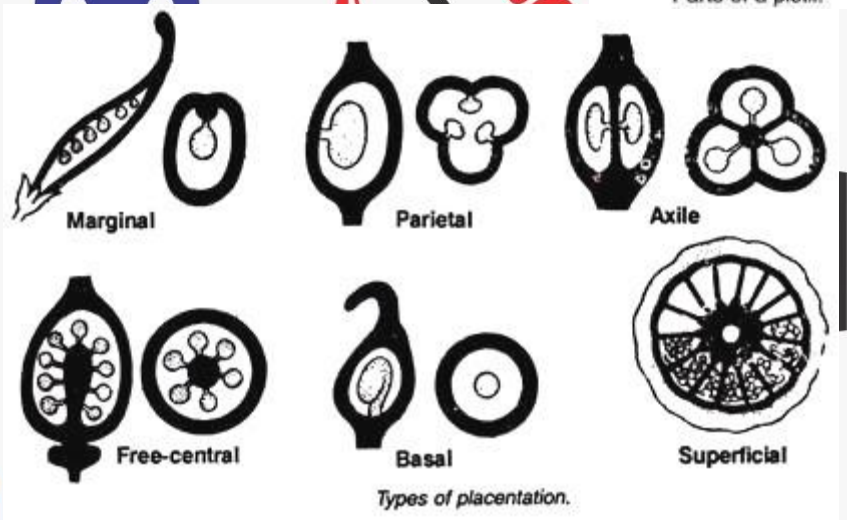
Parts of a pistil.

a. The Stigma: The stigma is the specialized part of the pistil on which the pollen grains are trapped during pollination. Stigma is the terminal receptive part of the pistil which functions as landing platform for the pollen grains and aid its germination. It also determines the compatibility-incompatibility of the pollen grains. There is considerable variation in the nature of the stigma, and accordingly it may be of the wet or the dry type. These two basic types based on the amount of secretion present during the receptive period.

b. The Style: The style is a elongated narrow tubular structure that connects the stigma with the ovary and is mainly of two types, viz., solid (closed) and hollow (open). The solid style has a central stand of transmitting tissue of elongated cells interconnected by plasmodesmata.

c. Ovary: it is the basal swollen part of the pistil. It has an ovarian cavity with one or more chambers or locules and ovule bearing parenchymatous cushions called placentae (singular placenta). An ovary may have one (e.g., Wheat, Paddy, Mango) to several ovules (e.g., Papaya, Water Melon, Orchids).

PLACENTATION- DEFINITION AND TYPES



Types of placentation.

- The portion of the carpellary tissue to which the ovules are attached is the placenta and their distribution in the ovary is described as placentation. or
- The arrangement or distribution of placentae in the cavity of the ovary is known as placentation.
- The most common types of placentation found in plants are as follows:

1.**Marginal placentation:**
- The placenta forms ridges along the ventral suture of the ovary.
- The ovules are borne on this ridge forming two rows. It occurs in monocarpellary and unilocular ovary.
- This is found in leguminous plants. Example: gram, peas, beans.

2.**Parietal placentation:**
- The placenta is situated on the meeting of two margins of subsequent carpels which may be two or more in number.
- In this type of placentation, the ovules develop on the inner wall of the ovary.
- Ovary has a single chamber but appears to be double-chambered because of the formation of a false septum called **replum**. It occurs in bicarpellary or multicarpellary but unilocular ovary, e.g., Papaveraceae, Cucurbitaceae- Cucumber, Muskmelon, Crucifereae- Mustard and *Argemone***.**

3.**Axile placentation:**
- Ovules are borne at or around the center of a multi-chambered ovary (multilocular) on an axis formed from joined septa.
- It occurs in bi-to multilocular ovary, e.g., Malvaceae, Rutaceae- Lemon, Orange, Solanaceae. Example: lady's finger, tomato.

4.**Basal placentation:**
- The placenta is situated at the base (bottom) of the ovary and a single ovule is borne on it.

- This is found in plants of the family Compositae. Example: sunflower, mariegold.

5.**Free central placentation:**

- Here, the placenta develop in the centre of the ovary as a prolongation of floral axis and the ovules are attached on this axis. It occurs in multicarpellary but unilocular ovary, e.g., Primulaceae- *Primula* (Primrose).

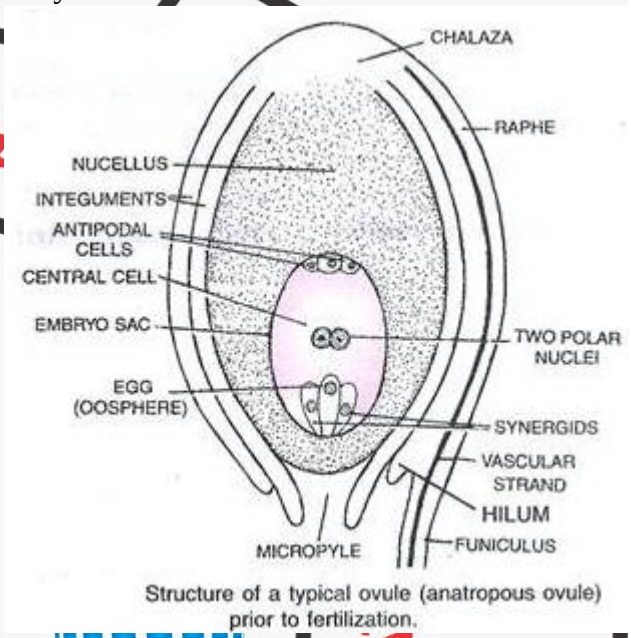
6.**Central placentation:**

- The ovules develop from the central axis of the multi-locular ovary.
- The ovary looks unilocular due to the breaking of partition walls.
- Example: Dianthus (family Caryophyllaceae).

7.**Superficial placentation:**

- Ovules arise from the inner wall of the septa in a multilocular ovary.
- Example: Water lily, Lotus (family Nymphaeaceae).

STRUCTURE OF MEGASPORANGIUM (OVULE):



Structure of a typical ovule (anatropous ovule) prior to fertilization.

The ovule in a flower is an integumented mega-sporangium within which the meiosis and megaspore formation takes place, which develops into a seed after fertilisation. An angiosperm ovule is typically an ovoid and whitish structure. It occurs inside ovary where it is attached to a parenchymatous cushion called placenta either singly or in a cluster. Each ovule usually consists of a nucleus invested by one or two integuments and a stalk called funiculus or funicle. In the typical (anatropous) ovule the junction between an ovule and a funicle which is fused with body of the ovule lengthwise beyond is called hilum, which later becomes a scar on the seed. It gives rise to a longitudinal ridge called raphe. Funiculus contains a vascular strand for the supply of nourishment to the ovule.

The body of the ovule consists of a mass of parenchymatous cells named nucellus. It is equivalent to mega sporangium. Cells of the nucellus are rich in reserve food materials. Nucellus may be quite massive (crassinucellate ovule) or thin (tenuinucellate ovule). It is surrounded by one (unitegmic ovule, e.g., higher dicots) or two (bitegmic ovule, e.g., monocots and primitive dicots) multicellular integuments. Rarely an ovule may be surrounded by three integuments (tritegmic, e.g., *Asphodelus*) or the integuments are absent (ategmic, e.g., *Santalum*). Free surfaces of nucellus and integuments are covered by cuticle. The integuments leave a narrow pore or passage at one end of the ovule. It is known as micropyle. The basal part of an ovule just opposite to micropyle or the place of origin of the integuments usually lies at the opposite end. It is termed as chalaza.

There is generally a single embryo sac or female gametophyte located in the micropylar half of the nucellus.

Integuments (endothelium): In most of the plants belonging to the sympetalae with unitegmic, tenuinucellate ovules, the nucellus degenerates at an early stage of ovule development and the innermost layer of the integument becomes specialized to perform the nutritive function for the embryo sac. This specialized tissue, present around the embryo sac, is called endothelium. The differentiation of endothelial layer is also known in some plants with bitegmic ovules. In these cases it is derived from the inner epidermis of the inner integument. It may differentiate even before the disorganization of the nucellus. The occurrence of endothelium has been observed in 65 families of dicots. In 47 families it forms a diagnostic feature.

The endothelium is usually single layered. In Asteraceae it may become multilayered: ten to twelve layered Ex: Sunflower. The cells of endothelium are radially elongated and rich in cytoplasm and store starch and fats. They often become polyploid.

The endothelial cells are separated from the nucellus by two layers of cuticle, one of nucellus and other of integuments. The cuticular layers later fuse. In addition to the cuticle, the cells of the endothelium also show wall thickenings. Normally, the endothelium covers the entire embryo sac. However sometimes it may remain confined to the lower two thirds of the chalazal half or to the micropylar end of the embryo sac. The time of differentiation

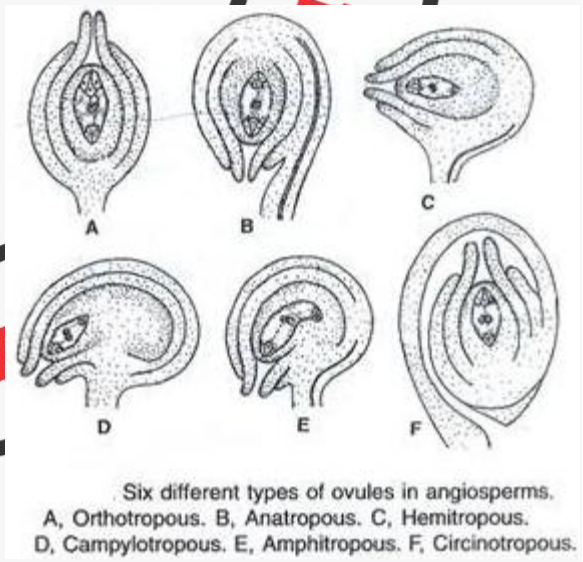
of endothelium and the duration of its activity are also variable. In some families the endothelium persists as a distinct layer in the seed. It forms a pigment layer in Polemoniaceae, Plantaginaceae and Linaceae.
At the ultrastructural level the endothelial cells exhibit features characteristic of meristematic and secretory cells. They possess high concentrations of proteins, RNA, carbohydrates, ascorbic acid and enzymes. The endothelila cells are in communication with each other and with those of the integument through plasmodesmata.
An interesting feature of the endothelial cells is the development of adventive embryos.
Although detailed structural and physiological studies concerning endothelium are lacking, its presence around the embryo sac and its cytological features suggest that it may be functionally similar to the anther tapetum which surrounds the sporogenous tissue. For this reason endothelium is also called integumentary tapetum.
In the Begoniaceae, Droseraceae, Elatinaceae and Fabaceae the persistent nucellar cells form an endothelium like tissue. Because of its different origin it has been called false endothelium.

Micropyle: Depending upon the presence or absence of integuments, the micropyle may or may not be organized. In bitegmic ovules the micropyle is generally formed by either both the integuments or only the inner integument. Only rarely does the outer integument alone constitute the micropyle. When both the integuments are involved the passage formed by the outer integument is called exostome and that by the inner integument is called endostome. The exostome and endostome may be in same line or not. The micropyle will be filled with an exudate given out by the nucellar cap and the inner integument. An interesting feature is the formation of a thin sheet of material across the exostome for sealing. The exudate and the thin sheet ensure a localized deposition of synergid-synthesized chemotropic agents in the micropyle. This may act as a stimulus for the pollen tube to enter the micropyle.

Obturator : Any ovular structure associated with directing the growth of the pollen tube toward the micropyle is generaly referred to as obturator. Obturators exhibit great variation in their origin, morphology, anatomy and extent of development. They may originate from placenta or funiculus, or both. The most common type of obturator is one formed by local swelling of the funiculus (Acanthaceae, Anacardiaceae, Lamiaceae, Magnoliaceae). Placental obturator occurs in the Euphorbiaceae and Cuscutaceae.

The obturator may fill the micropyle to varying degrees. The pollen tube grows along the obturator. The cells of the obturator produce a surface exudate and provide nutrition, mechanical and chemical guidance to the pollen tube.

Nucellus (crassinucellate and tenuinucellate conditions).
TYPES OF OVULE:
Depending upon the configuration and orientation of the body of ovule in relation to funiculus, there are six types of ovules—
1. Orthotropous or atropous ovule (ortho-straight, tropous - turn)
The body of the ovule is erect or straight. The hilum, chalaza and the micropyle lie in a straight line e.g.***Polygonum*.**
2. Anatropous ovule (ana - backward or up, tropous - turn)
The body of the ovule becomes completely inverted during the development so that the micropyle lies very close to the hilum (eg) Gamopetalae members.
3. Hemi-anatropous or hemitropous ovule
The body of the ovule is placed transversely at right angles to the funicle. The micropyle and chalaza lie in one straight line e.g.***Ranunculus*.**

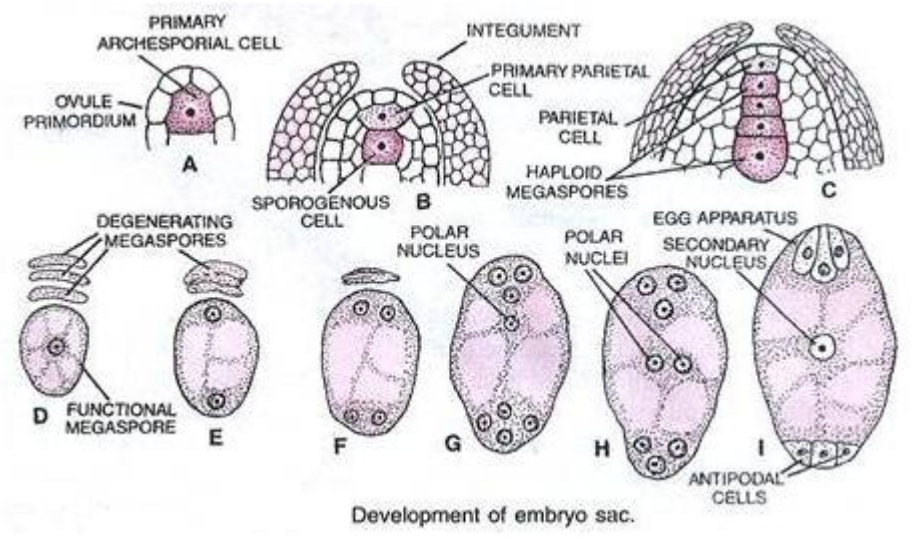


Six different types of ovules in angiosperms.
A, Orthotropous. B, Anatropous. C, Hemitropous.
D, Campylotropous. E, Amphitropous. F, Circinotropous.

4. Campylotropous ovule (kampylos - curved)
The body of the ovule is curved or bent round so that the micropyle and chalaza do not lie in the same straight line. e.g. ***Leguminosae*.**
5. Amphitropous ovule The curvature of the ovule is very much pronounced and the embryosac also becomes curved e.g.***Alismataceae,*** and ***Butamaceae*.**
6. Circinotropous ovule

The nucellus and the axis are in the same line in the beginning but due to rapid growth on one side, the ovule becomes anatropous. The curvature continues further and the micropyle again points upwards (e.g.) ***Opuntia*.**

MEGA-SPOROGENESIS (DEVELOPMENT OF AN OVULE):



Development of embryo sac.

Ovule develops as primordium and then mound of nucellus over placenta. Initials of integuments develop from its base. They grow and come to surround the nucellus on all sides except at the tip or micropylar region. In the hypodermal region of nucellus towards the micropylar end develops a primary archesporial cell. It grows in size and develops a prominent nucleus.

The archesporial cell often divides once into outer primary parietal or wall cell and inner primary sporogenous cell. Primary parietal cell may divide one or more times. The primary sporogenous cell commonly functions directly as diploid megaspore mother cell or megasporocyte. The megaspore mother cell (MMC) enlarges in size and undergoes meiosis to form a linear tetrad of 4 haploid megaspores. The process of meiotic formation of haploid megaspores from diploid megaspore mother cell is called megasporogenesis. Commonly the chalazal megaspore remains functional while the other 3 degenerate.

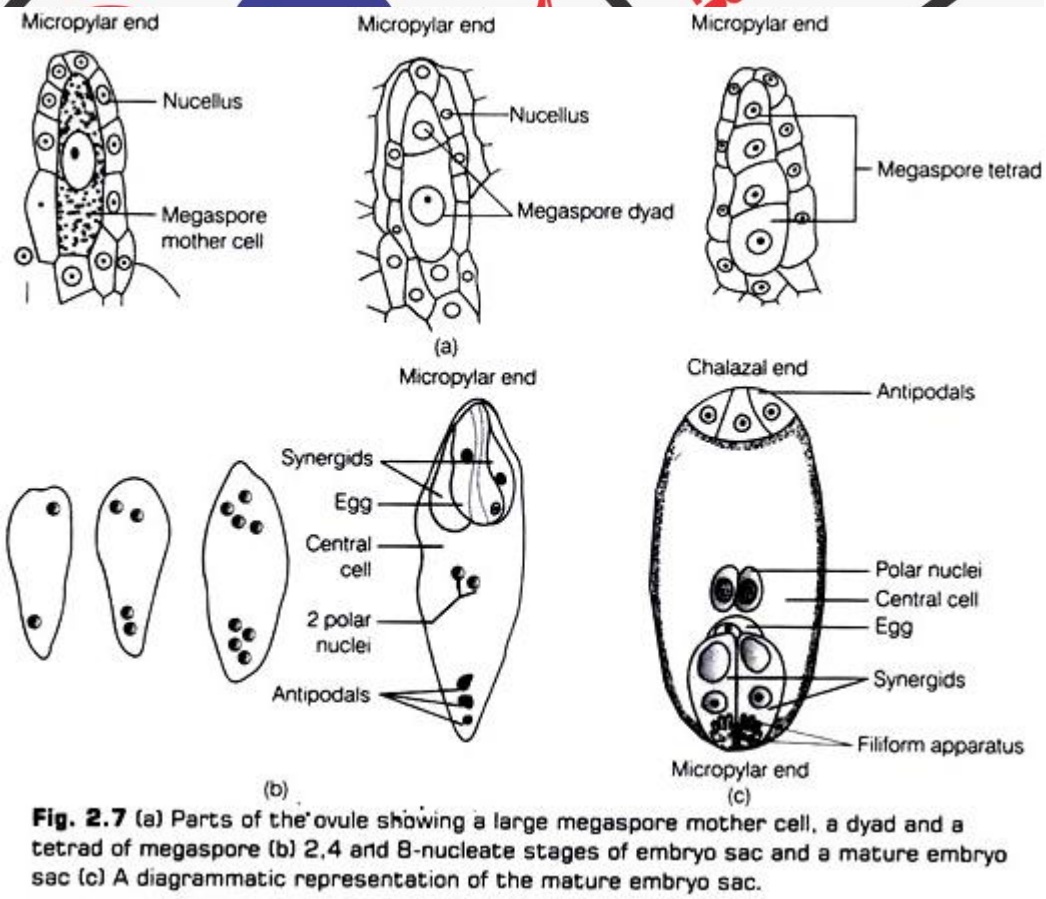


Fig. 2.7 (a) Parts of the ovule showing a large megaspore mother cell, a dyad and a tetrad of megaspore (b) 2,4 and 8-nucleate stages of embryo sac and a mature embryo sac (c) A diagrammatic representation of the mature embryo sac.

It occurs inside the nucellus of developing ovule of angiosperms. The process begins very early when nucellus is not completely surrounded by the integuments.

Development of Female Gametophyte (Mega-gametogenesis):

Following are the different stages of development of female gametophyte:
(i) The functional haploid megaspore is the first cell of female gametophyte of angiosperm.
(ii) It enlarges in size to form the female gametophyte, also called embryo sac.

(iii) Its nucleus undergoes a mitotic division and the two nuclei move to the opposite poles, forming the 2-nucleate embryo sac.
(iv) The 2-nucleate embryo sac undergo second mitotic division giving rise to the 4-nucleate stage.
(v) The third mitotic division gives rise to 8-nucleate embryo sac, which comprises of a micropylar end and a chalazal end with four nuclei at each end.
(vi) Out of four nuclei at the micropylar pole, 3 differentiates to produce an egg apparatus consisting of 2 synergids and a female gamete egg cell.
(vii) Similarly, at the chalazal end, 3 out of 4 nuclei are grouped together and are surrounded by cytoplasm and cellular wall differentiate as antipodal cells.
(viii) The remaining nuclei, one at the micropylar end and one at the chalazal end termed as polar nuclei migrate towards the center of the embryo sac (now called central cell). They meet at the center and may remain separate until the discharge of male gametes take place and then fuse to form secondary nucleus of central cell.
(ix) Thus, a typical angiosperm embryo sac is 8-nucleate and 7-celled.

This type of embryo sac development is very common in angiosperms and is known as ordinary type or normal type or Polygonum type. This type is also known as monosporic type, because, out of four megaspores, only one megaspore situated towards chalazal end remains functional and forms the embryo sac, while the remaining three megaspores gradually degenerate and finally disappear.

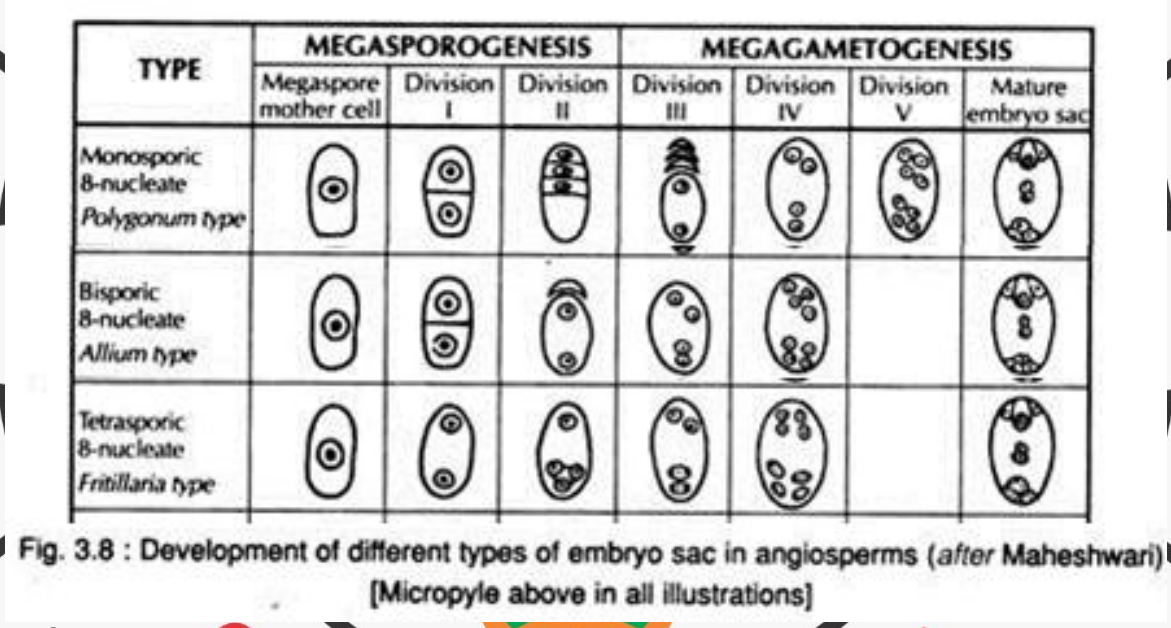


Fig. 3.8 : Development of different types of embryo sac in angiosperms (*after* Maheshwari)
[Micropyle above in all illustrations]

Other types of embryo sac development

Bisporic type: Allium type:

The megaspore mother cell divides to form two cells, the upper one quickly degenerates. The lower one then divides and forms two nuclei, distributed in the two poles. Later on, both the nuclei undergo two successive divisions and form usual octant type of embryo sac, i.e., *Polygonum* type. Here two megaspore nuclei take part in the development of embryo sac i.e., bisporic type, e.g., *Allium, Scilla, Trillium* etc., of Liliaceae.

Tetrasporic type: Fritillaria type:

The megaspore mother nucleus undergoes meiotic division and forms four nuclei which remain crosswise in the embryo sac without any wall. Out of four nuclei formed, one nucleus remains towards the micropyle, and the rest three at the chalazal end. The chalazal nuclei fused together and form 3n nucleus. Both the cells thus undergo one mitotic division and again form a tetrasporic stage. Out of four nuclei, two remain at each pole.
All the nuclei then undergo mitotic division and form eight nuclei. Out of four haploid nuclei at the micropyle, one egg and two synergids are formed, those remain at the micropylar end; three triploid nuclei at the chalazal end and one from each pole remain at the centre (one haploid and the other one triploid), e.g., *Fritillaria, Tulipa* and some other members of Liliaceae.

STRUCTURE OF MATURE EMBRYO SAC (FEMALE GAMETOPHYTE):

In angiosperms, the female gametophyte is called embryo sac. Embryo sac is an oval multicellular haploid structure which is embedded in the nucellus towards micropylar half of the ovule. It is covered over by a thin membrane derived from the parent megaspore wall. The typical and most common type of embryo sac, found in 80% flowering plants is *Polygonum* type (Fig. 2.15).

It contains 8 nuclei but 7 cells— 3 micropylar, 3 chalazal and one central. It is formed by one meiosis (formation of 4 megaspores from one MMC) and three mitosis (inside functional megaspore). The three micropylar cells are collectively known as egg apparatus (equivalent to one archegonium).

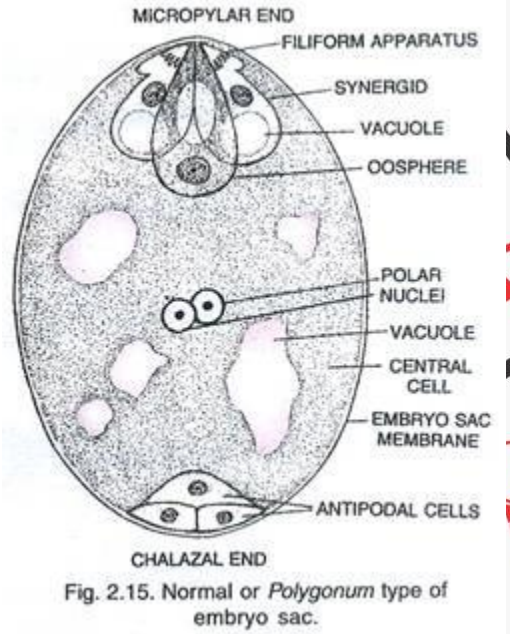


Fig. 2.15. Normal or *Polygonum* type of embryo sac.

They are pyriform in outline and are arranged in a triangular fashion. The three cells of egg apparatus have conspicuous common walls towards micropylar half. They separate and become thin towards the central cell.

One middle cell is larger and is called egg or oosphere. It has a central or micropylar vacuole and a nucleus towards the chalazal end. A filiform apparatus may or may not be present. The remaining two cells are called synergids, cooperative cells or help cells. Each of them bears a filiform apparatus in the micropylar region, a lateral hook, chalazal vacuole and a central nucleus.

A filiform apparatus is a mass of finger like projections of the wall into the cytoplasm. In embryo sac, one synergid degenerates at the time of entry of pollen tube into the embryo sac, whereas, the second one degenerates shortly after the embryo sac has received the pollen tube discharge.

All the three cells of the egg apparatus communicate with one another and to the central cell by plasmodesmata. The egg or oosphere represents the single female gamete of the embryo sac. The synergids help in obtaining nourishment from the outer nucellar cells, guide the path of pollen tube by their secretion and function as shock absorbers during the penetration of pollen tube into the embryo sac.

The three chalazal cells of the embryo sac are called antipodal cells. They are the vegetative cells of the embryo sac which may degenerate soon or take part in absorbing nourishment from the surrounding nucellar cells. Internally they are connected with the central cell by means of plasmodesmata. Antipodals possess a definite cell wall. In some embryo sac antipodals are completely absent. The cytoplasm of the antipodals cells is rich in mitochondria, plastids and dictyosomes.

The central cell is the largest cell of the embryo sac and is considered as the mother cell of the endosperm. Large central vacuole is present in the central cell which has reserve food (**reservoir of amino acids, inorganic salts and sugars)** and Golgi bodies which is used up during the fertilization and endosperm formation. In the middle, the cell contains two polar nuclei which have large nucleoli. The polar nuclei often fuse to form a single diploid secondary or fusion or definitive nucleus. Thus all the cells of the embryo sac are haploid except the central cell which is first bi-nucleate and then becomes diploid due to fusion of polar nuclei.

Egg *Cell*

The egg cell is located at the micropylar end of the embryo sac and ultimately fuses with a sperm nucleus to produce a zygote. The egg cell lies adjacent to the two synergids, separated from them by either partial cell walls or the plasmalemma alone. The distribution of cytoplasm within the egg cell is highly polarized, due to the presence of a large vacuole at the micropylar end that restricts the nucleus and most of the cytoplasm to the chalazal end.

Synergids

The synergids are elongated cells which are located on either side of the egg cell at the micropylar end of the

embryo sac. When two synergids are present they lie in contact with each other and partly embrace the egg. They are pointed or hooked toward the micropyle.

The wall around the synergids is incomplete. There is a distinct wall around the micropylar one-third of the cell which thins toward the chalazal end and finally disappears. As a result, the chalazal one-third of the cell lacks a wall. In this region the protoplast of the synergid is separated from that of the central cell by double membrane; one of the synergid and the other of the central cell.

A prominent structure, called filiform apparatus (FA), is present at the micropylar end of the synergid. Its differentiation from the synergid wall also exist. Electron microscopic studies have revealed that the filiform apparatus is a mass of finger like projections of the wall into the cytoplasm. Cytochemical studies suggest that it is made up of a number of substances, including cellulose, hemicellulose, pectin, callose and proteins. Structurally, each projection of the filiform apparatus has a core of tightly packed micro fibrils (possibly cellulosic) enclosed by a non-fibrillar sheath. They are rich in polysaccharides. The form of filiform apparatus is variable from spherical, wedge-shaped and may be irregularly present all along the entire length of the synergids. The presence of abundant starch grains in a young synergid and their virtual absence from a fully developed synergid suggest that FA "seems to be formed mainly from substances transformed from starch grains".

The cytoplasm of the synergid is strongly polarized. The chalazal region of the cell is occupied by one large or many small vacuoles. Large amount of cytoplasm and a prominent nucleus are present in the micropylar half of the cell. The cytoplasm is rich in mitochondria, endoplasmic reticulum, dictyosomes, lipids, RNA and proteins are also abundant and are concentrated near the filiform apparatus with few plastids.

Synergids are ephemeral structures. In embryo sacs with two synergids, one degenerates before or soon after the entry of the pollen tube into the embryo sac, whereas the other one, often called the persistent synergid, degenerates shortly after the embryo sac has received the pollen tube- discharge. Occasionally, however one of the synergids may persist for a considerable period after fertilization. Its nucleus may enlarge and show polyploidy. As a rule, the synergids remain within the confines of the embryo sac. In some they may break through the embryo and project into the micropyle. One of the synergids becomes swollen and haustorial in nature.

FUNCTIONS OF SYNERGIDS

Central Cell

Positioned in the center of the embryo sac, this cell contains two nuclei, a large vacuole, and many cytoplasmic organelles. The polar nuclei originate at both the micropylar and chalazal ends of the coenocytic megagametophyte and migrate to the center after cellularization. The polar nuclei may partially fuse with each other before they are fertilized by a single sperm nucleus, generating the triploid primary endosperm nucleus. The mature endosperm will provide nutrients for the developing embryo or seedling.

Antipodal Cells

Three antipodal cells are located opposite the egg at the chalazal end of the embryo sac. No specific function during reproduction has been attributed to the antipodals, but they are thought to be involved in the import of nutrients to the embryo sac. Cytological characteristics of cells within the embryo sac as well as cytochemical localization of proteins, starches, lipids, and nucleic acids have been used to assess the physiological state of the embryo sac and suggest relative rates of metabolic activity. For example, the presence of numerous ribosomes and mitochondria in the synergids, central cells, and antipodals suggests a high metabolic activity. By contrast, the egg cell has fewer ribosomes, plastids, and other organelles and appears to be relatively quiescent.

Double fertilization – pollen germination

Pollen-Pistil Interaction:

Pollen grains are deposited on the stigma either due to closeness of the anthers to the stigma or by pollinating agents (biotic or abiotic). This unique feature brings about pollen-pistil interaction between the male gametophyte, the pollen grains, with the massive sporophytic tissue.

A successful pollination brings about sequential events in the pollen-pistil interaction that ultimately ends up by the discharge of the male gametes in the embryo sac (Fig 6.1).

Pollination → Pollen adhesion → Pollen hydration → Pollen germination → Pollen tube entry into stigma → Pollen tube growth through the style → Pollen tube entry into the ovule and embryo sac → Double fertilization

Fig. 6.1 : Stepwise events in pollen-pistil interaction

All the events beginning from pollination to the release of gametes in the embryo sac form a part of the pollen-pistil interaction or the programic phase.

Recognition of Compatible Pollen:

The pistil has the ability to recognise the right compatible pollen of the same species and to reject the pollen grains that are incompatible either of same species or of other species. After recognition of correct pollen pistil promotes post-pollen events that leads to fertilisation.

Fusion of the male gamete with the female gamete to form a diploid zygote within the embryo sac is called fertilisation. In angiosperms, the male gametes are carried to the egg by the pollen tube, termed as siphonogamy.

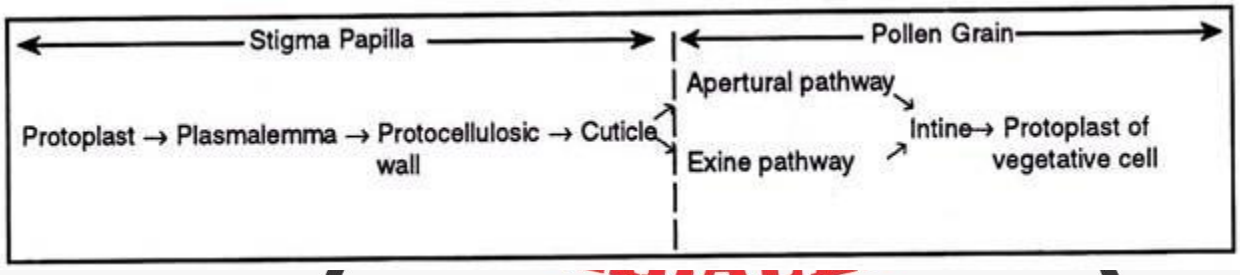
POLLEN ATTACHMENT AND HYDRATION:

The attachment of the pollen on the stigma depends upon its wall sculpture and stickiness. In wet stigma adhesion is mostly a mechanical process, whereas, in dry stigma it depends on the extent and composition of the pellicle, and the amount of surface-coat substances on the pollen.

Pollen hydration proceeds in a controlled manner characterized by distinct area of stability of increasing water content and can begin in the anther before pollen release. Its rapidity is dependent to a great extent on the nature of stigma, for instance in a dry stigma hydration is gradual and controlled by the water potential of the stigma and pollen.

This controlled hydration provides suitable conditions for the recovery of the membrane integrity of the vegetative cell.

A plausible pathway for hydration in dry stigma as proposed by J.Heslop-Harrison (1979) is given below:



In a stigma with aqueous exudates hydration is very rapid. For instance in *Petunia* the stigma is covered with a lipoidal exudates and a thin layer of water which establishes a moisture gradient through the lipoidal exudates. The pollen grain thus gradually gets hydrated.

Ultrastructural and physiological studies of pollen hydration in *Brassica* show two distinct phases of hydration. During the initial phase, putative signals are reciprocally exchanged between pollen and stigma. The second phase proceeds with an invagination of the intine in the colpial zone and formation of a 'foot' of pollen coating that contact the stigma papilla.

Freeze-etch preparation show microchannels at the papilla-pollen boundary through which water moves from stigma to pollen grain but not between grains. The area around the site of pollen tube emergence is rich in pectins, and one of the earliest visible alterations of macromolecules upon hydration is a loss of protein and pectic material from the length of the colpial slit.

POLLEN GERMINATION AND TUBE GROWTH:

The stigmatic surface provides the essential prerequisites for a successful germination that are absent in the pollen. In wet stigma, the role of the stigmatic exudates in pollen germination is highly variable.

In *Amaryllis* and *Crinum*, stigmatic exudates are essential for pollen germination, however, in *Nicotiana* and *Petunia* the exudates play no significant role during germination, since young stigmas free from exudates support satisfactory germination of pollen grains.

In dry stigma, the pellicle plays a vital role in germination.

The stigmatic surface also provides boron and calcium which are required for germination but are deficient in pollen. It has been seen that those stigmatic secretion of *Vitis vinifera* that contain 2-5 ppm of boron permit pollen germination.

The growth of the pollen tube of flowering plants is restricted exclusively at their apices. Microscopic examination of growing pollen tubes reveals that most of the cytoplasm is restricted to the apical region while a large vacuole fills the grain and the older region of the tube (Figure 6.6). The cytoplasm is restricted to the apical region of the growing tube by the formation of series of callose plugs at regular intervals behind the tip (Figure 6.7).

The callose plugs are formed as a ring on the inner side of the tube wall and gradually grow toward the centre which finally seals off the growing tip from rest of the pollen tube. There is characteristic zonation, in which the apical region of the tube possesses a clear cap called **"cap block"** with more granular

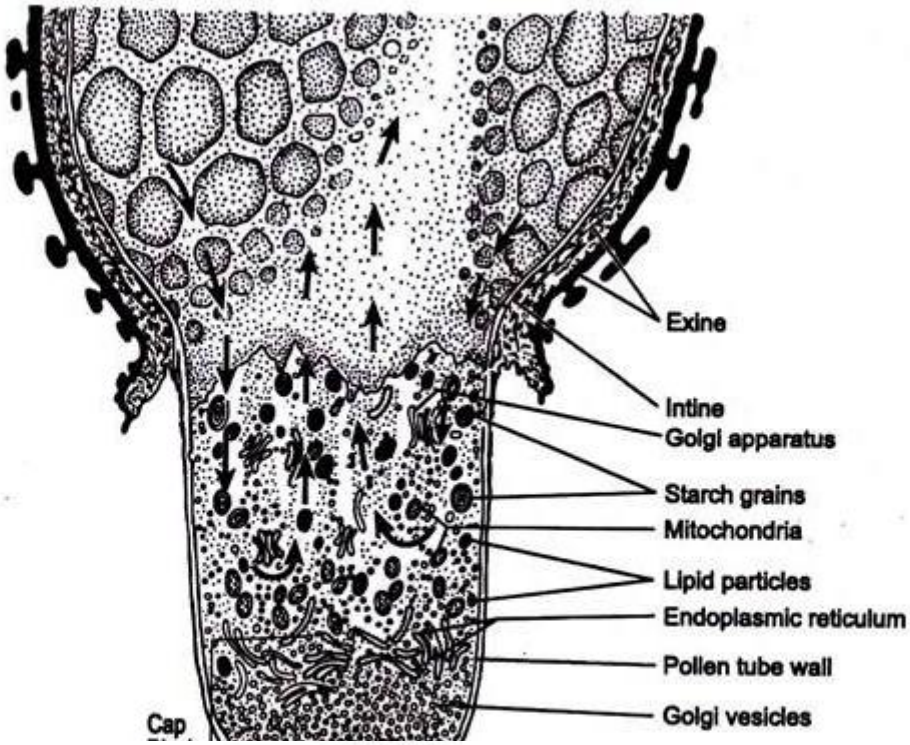


Fig. 6.6 : Fine stru
vesicles, ER, Golg

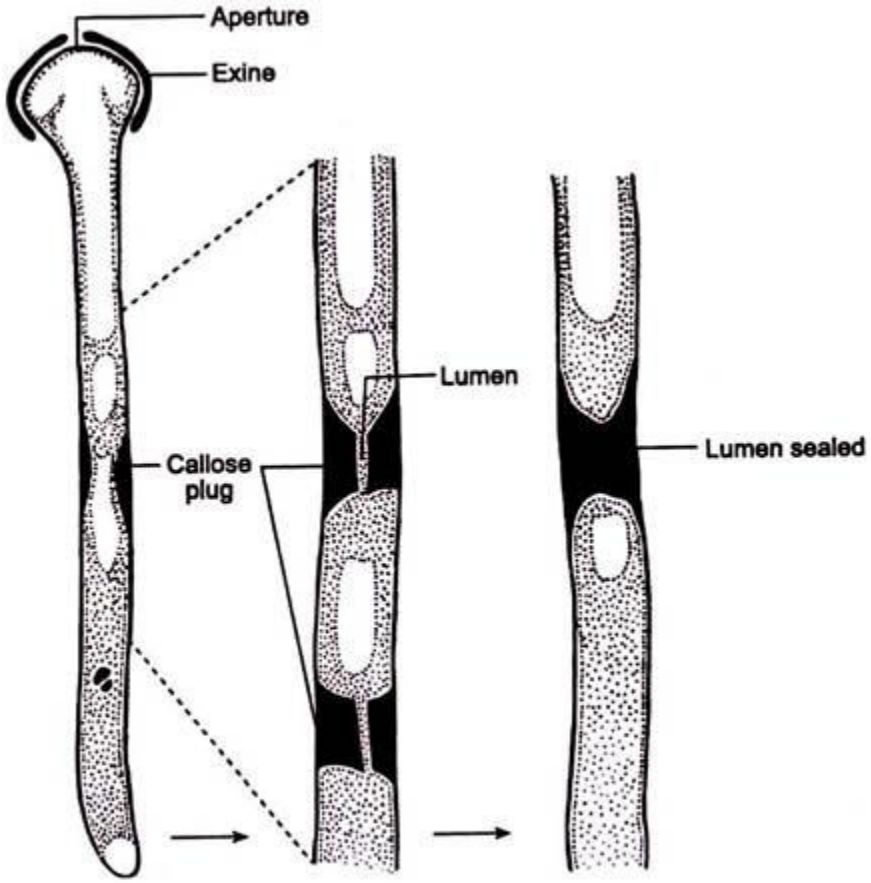


Fig. 6.7 : Different stages of callose plug formation in a growing pollen tube.

elements behind. The "cap block" disappears with the termination of the growth. These internal components exhibit vigorous "reverse fountain" cytoplasmic streaming.

However, within the tip itself the motion is chaotic and turbulent, with vesicles appearing to move in a random, from the base of the clear zone to the extreme apex. There is marked accumulation of secretory vesicles often in the shape of an inverted cone at the tube apex.

These vesicles contain components for cell wall expansion, because more vesicles are secreted than are required to support the increased area of the plasma membrane. Actin polymerization is necessary for pollen tube growth.

Actin microfilaments (MFs) are involved in the transport of secretory vesicles essential for cell elongation and is accumulated in substantial amounts in mature pollen grains. The cytoplasm behind the tip is rich in cell organelles, lipid bodies, vesicles, and amyloplasts.

Pollen tubes do not grow uniformly, but rather in bursts or pulses. In *Petunia* and *tobacco*, the tube cell elongates with alternating bursts of fast and slower growth.

Many underlying physiological processes also oscillate with the same frequency, but with varying phase relationships to growth rate. For example, the intracellular Ca^{2+} gradient oscillates in phase or slightly behind the growth peak, while the extracellular Ca^{2+} influx exhibits a 10-15 sec delay. H^+ also oscillates.

To date, genes specifically associated with pollen germination have not been identified. However, the large number of unidentified proteins (> 230) whose appearance is coincident with germination suggests that it may be premature to conclude that none of them arises from transcripts activated specifically at germination.

Some early gene products such as alcohol dehydrogenase, actin, and a heat- shock protein from tomato persist in germinating pollen. Although the late genes are transcriptionally activated before dehydration, their persistence during germination and growth argues for a functional role at this stage.

A pollen specific calcium-dependent calmodulin-independent protein kinase (CDPK) isolated from maize suggests the presence of post-translational control mechanism involving Ca^{2+} and phosphorylation. The gene is transcribed in mature and germinating pollen and is required for germination.

Following pollen germination the pollen tube-grows on the surface of the stigmatic papillae, e.g., *Gossypium*, or through the cellulose-pectic layer of their walls, e.g., *Lilium*. The stigma provides the pollen with water and necessary medium in the form of exudates for its germination. The exudates are highly viscous, refractive and adhesive. These are rich in lipids, small amount of free sugars, amino acids, proteins, and peptides.

In dry stigma and solid style, the pollen tube degrades the cuticle by cutanase released by the pollen. Pollen grains contain an elaborate set of enzymes and some of these are available as soon as the pollen grain makes contact with the stigma.

The digestion of the cuticle allows the tube to enter the pectocellulosic wall of the papillae and finally grow through the intercellular substances of the stigma and the style. In *Gladiolus* the pollen tube grows through a mucilaginous substance accumulated between the cuticle and cell wall, instead of the pectocellulosic wall.

In wet stigma and solid style the cuticle gets disrupted during the secretion of the exudates and the pollen tube enters the intercellular matrix of the stigmatic tissue.

PATH OF POLLEN TUBE IN THE STYLE:

In species with wet stigma and solid style the cuticle of the stigma /papillae is disrupted during the secretion of the exudates, thus there is no physical barrier for pollen tube entry into the intercellular spaces of the transmitting tissue of the stigma. In taxa with wet stigma and hollow style, pollen tubes grow on the surface of the stigma and enter the stylar canal.

In species with dry stigma and solid style the cuticle provides the physical barrier for the pollen tube entry. The cuticle is eroded at the point of contact by the activity of cutinases released by the pollen. After the digestion of the cuticle, the tube enters the pectocellulosic wall of the papillae and finally grows through the intercellular substances of the stigma and the style.

In *Gladiolus* and *Crocus* the pollen tube grows through the mucilage accumulated between the cuticle and the cell wall.

Pollen tube growth is a calculated directional cell migration, along the transmitting tissue of the style. In most of the species pollen tube make their way to the ovary through the intercellular matrix of the transmitting tissue or through the mucilaginous matrix of the hollow style. The secretion product of the glandular cells of the solid stylar tissue is deposited in the matrix.

It is a heterogenous mixture consisting chiefly of sugars, proteins, glycoproteins, and lipids. In several dicotyledons and monocotyledons the transmitting tissue contains arabinogalactan proteins. This glycoprotein is style-specific,

and its presence in the cytoplasm and cell walls of compatible pollen tubes growing in the style have favoured its role in the nutrition, growth, and guidance of pollen tubes in the stylar tract.

In the hollow styles, the canal cells secrete a mucilaginous substance that later forms an extracellular matrix and accumulates in the stylar canal. The major component of this secretion is again an arabinogalactan protein.

PATH OF POLLEN TUBE INTO THE OVULE AND EMBRYO SAC:

After reaching the ovary, the pollen tube enters the ovule through micropylar end. It then enters one of the synergids through the filiform apparatus present at micropylar end. The synergids direct the growth of pollen tube by secreting some chemical substances.

The tip of the pollen tube enters one synergid, then the penetrated synergid starts degenerating. After penetration, the tip of the pollen tube. The pollen tube finally pushes through the ovule and reaches the embryo sac. This guidance into the ovule is in terms of essential signals originating from the male and female tissues.

Evidences obtained from the analysis of developmental mutants of *Arabidopsis*, viz., bell and sinl (where integument and embryo sac development in the ovule is affected) suggests that genes active in the female gametophyte play a crucial role in the signalling process that guides pollen tubes to the ovule.

In fact pollen tubes are always attracted to ovules with a functional embryo sac, which confirms a female gametophytic control of pollen tube guidance.

The principal significance of pollen-pistil interactions are:

a. The most essential requirement for sexual reproduction is the screening and selection of male gamete and this is achieved during pollen pistil interaction. Thus, pollen-pistil interaction offers enormous potential for the manipulation of pollen screening which is obviously for the quality and compatibility of pollen.

b. The number of pollen grains that are generally deposited on the stigma under normal conditions are far greater than the number of ovules available for fertilization. As a result the pollen grains are subjected to a tough competition during pollen-pistil interaction.

Only those pollen that germinate early and have a faster growing pollen tube, i.e., more vigorous, are able to withstand the rigidity of post- pollination competition and fertilization. Consequently competition among pollen grains during pollen-pistil interaction results in the increased vigour of the progeny. Thus this interaction can be considered as an important contributory factor in the evolutionary success of flowering plants.

c. It has a direct relevance to plant breeding programmes. A plant breeder continuously strives to bring together desirable characters present in different taxa, through hybridization.

Thus a better understanding of the biology of pollen-pistil interaction would no doubt, enable the plant breeders to manipulate the screening process in the pistil more effectively.

POLLEN TUBE DISCHARGE WITH MALE GAMETES (SPERMS):

(a) The pollen tube contains two sperms (each is a haploid male gamete).

(b) When pollen tube enters the embryo-sac (inside the ovule), it bursts to release its contents i.e., two sperms along with certain amount of protoplasm .The first male gamete discharge in one of the synergid.

DOUBLE FERTILIZATION:

(a) Both the male garnet present in pollen tube utilises in fertilisation process of angiosperm is known as double fertilisation. It is the characteristic feature of angiosperms except Family Orchidaceae, Podostemaceae and Trapaceae.

It involves two types of fusion –

a. Syngamy (fusion of egg cell and one male gamete) and

b. Triple fusion (fusion of remaining male garnet and two proper nuclei).

c .It was first observed by Nawaschin (1898) in Fritilaria and Lilium. It was supported by Guignard (1899).

(1) Syngamy:

(a) One of the two sperms goes to fertilize the egg cell. This fusion is called syngamy.

(b) It results in the formation of zygote, which gives rise to proper embryo.

(2) Triple fusion:

(a) The remaining sperm now fuses with the two haploid polar nuclei (present in the centre of embryo sac).

(b) This fusion is called as triple fusion (as three nuclei i.e., one male garnet and 2 polar nuclei, are fused).

(c) It results in the formation of triploid endosperm nucleus, which on development (Repeatd mitosis) form the endosperm.

(d) Endosperm is therefore triploid in angiosperms (It is a characteristic feature of angiosperms)

(e) Endosperm serves to provide nutrition to the developing embryo.

ENDOSPERM

Meaning of Endosperm:

The endosperm makes the main source of food for the embryo. In gymnosperms, the endosperm is haploid (n) and forms a continuation of the female gametophyte. On the other hand, in angiosperms it is formed mostly as the result of a fusion of the two polar nuclei and one of the male gametes. Since all the three nuclei taking part in the fusion are haploid, the endosperm becomes triploid (3n).

In normal cases, the endosperm is triploid but haploid, tetraploid and polyploid endosperms are also known. Generally the endosperm nucleus divides after the division of the oospore, but in several cases the endosperm is formed to a great extent even before the first division of the oospore. However, endosperm formation is suppressed in two angiospermic families, the Orchidaceae and Podostemonaceae. There are two types of seeds for storage of food:

b) Endospermic or albuminous seed: The endosperm supply food to the developing embryo. Such seeds are called endospermic seeds. In plants like corn, wheat, the endosperm tissue is present in the time of seed germination. So these are endospermic seeds.

c) Non–endospermic or ex–albuminous seeds: In some cases, the endosperm is completely utilized by developing embryo. Such seeds are known as non-endospermic seeds. In beans and peas the endosperm tissue is completely digested by the developing embryo and stored in the cotyledons.

Structure of Endosperm

The cells of the endosperm are isodiametric. They store large quantity of food materials. The storage food is present in the form of starch granules, granules of proteins, or oils. In certain plants, the endosperm cells develop very thick hard walls of hemicelluloses. The parietal layer of the endosperm of grass functions like a cambium. This layer produces on its inside layers of thin-walled cells. These cells are packed with starch. The cells of outermost layer stops dividing. It is filled with aleurone grains. This layer is called **aleurone layer.** The cells of this layer secrete diastase and other enzymes. These enzymes digest the food stored in endosperm for developing embryo.

TYPES OF ENDOSPERM FORMATION:

There are three general types of endosperm formation:

(a) Nuclear type,
(b) Cellular type and
(c) Helobial type.

FREE NUCLEAR TYPE (Areca catechu, Cocos nucifera**):**

In this type, the first division and usually several of the following divisions are unaccompanied by wall formation. The nuclei may either remain free or in later stages, they may become separated by walls.

As divisions progress, the nuclei are being pushed towards the periphery, thus a large central vacuole is formed. Often the nuclei are especially aggregated at the micropylar and chalazal ends of the sac and form only a thin layer at the sides.

Generally the endosperm nuclei in the chalazal part of the embryo sac have been observed to be larger than those in the micropylar end. The number of free nuclear divisions varies in different plants.

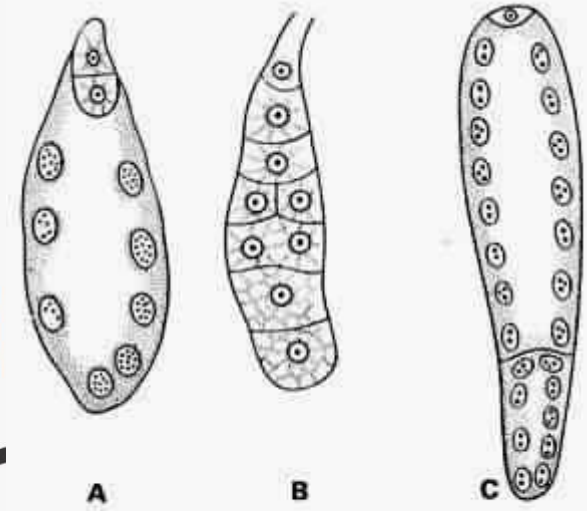


Fig. 46.39. Development of endosperm. A, nuclear type; B, cellular type and C, helobial type.

The development of the endosperm of *Cocos nucifera* of Palmae deserves special mention. Here the primary endosperm nucleus undergoes a number of free nuclear divisions. When the fruit is about 50 mm long the embryo sac remains filled with a watery fluid or milk containing free nuclei and fine cytoplasmic particles.

At a later stage when the fruit becomes about 100 mm in length the liquid shows in addition to free nuclei, several cells each enclosing variable number of nuclei. Gradually these cells and free nuclei set at the periphery of the cavity, and layers of cellular endosperm are formed, and this becomes the coconut meat.

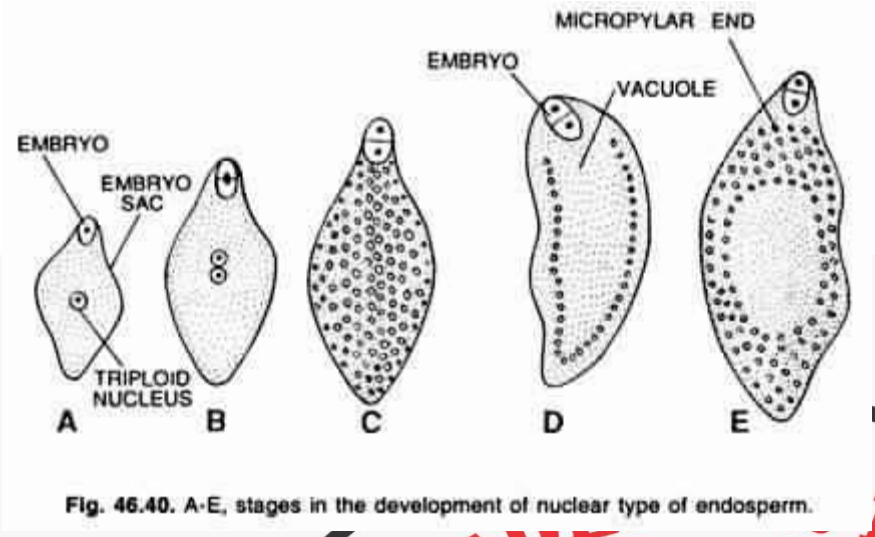


Fig. 46.40. A-E, stages in the development of nuclear type of endosperm.

On maturity of coconuts the endosperm does not have free nuclei or cells. In *Areca nut* the development of the endosperm is like that of coconut but the embryo sac cavity is small and it is completely filled up by the growth of the endosperm, and later becomes very hard.

The nuclear type of endosperm formation is the most common type and found in maize, wheat, rice, sunflower, etc.

CELLULAR TYPE (*Cucumis*):

In this type, the first and most of the following divisions are accompanied by wall formation and thus the sac is divided into several chambers, some of which may contain more than one nucleus. The first wall is usually transverse but sometimes vertical or oblique, and in some other cases, the plane of division is not constant.

On the basis of the orientation of walls following the first two or three divisions, this type of endosperm has been further divided into several subtypes.

HELOBIAL TYPE:

This type is frequently found in the members of the order Helobiales. This type is intermediate between the nuclear and the cellular types. In this type the first division is followed by a transverse wall resulting in a micropylar and chalazal chamber. Further divisions are generally free nuclear and may be formed by the micropylar chamber only.

Eremurus is an example of a typical Helobial endosperm. Here the primary endosperm nucleus divides tranversely forming two chambers, a large micropylar and a small chalazal. Free nuclear divisions occur in both but are more rapid in micropylar chamber. Thus, when four nuclei are formed in the chalazal chamber, eight nuclei are produced in the micropylar chamber.

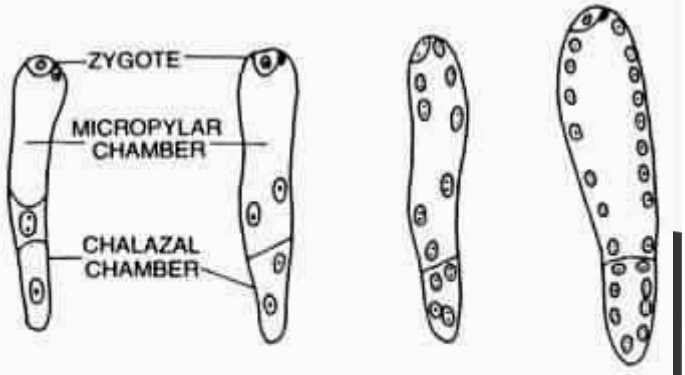


Fig. 46.41. Development of helobial type of endosperm in *Eremurus*.

When the chalazal chamber has eight nuclei, the micropylar chamber contains sixteen nuclei, and when there are 30 to 32 nuclei in chalazal chamber the micropylar chamber has considerably a large number of nuclei.

In older ovules, the chalazal chamber begins to degenerate. Finally, when cell formation takes place in the micropylar chamber, the chalazal chamber is almost crushed and shows only a few disorganized nuclei.

RUMINATE ENDOSPERM:

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

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

Mosaic Endosperm

Endosperm containing tissues of two different types is called mosaic endosperm. It occurs in plants like corn. In this case, endosperm lack of uniformity in the tissues. The endosperm contains patches of two different colours. It forms a sort of irregular mosaic pattern. The part of endosperm is starchy and part is sugary.

Perisperm

In this case, a part of nucellus may persist in embryo in the form of an apical cap. It acts as a nutritive tissue and called perisperm. It occurs in some dicots such as pepper and water-lily.

EMBRYOLOGY OF ANGIOSPERMS III

Embryogenesis in Dicot (*Capsella bursa-pastoris)*

The development of *Capsella bursa-pastoris* (Shepherd's purse) embryo is taken as model organism for the study of development of embryo of dicots. Following developmental changes take place in the embryo *Capsella bursa-pastoris.*

First division of Oospore: Its oospore increases in size. It divides transversely in two cells. The cell toward the micropyle end is called **suspensor cell.** The cells towards other side is called **embryonal cell.** Embryonal cell forms the major portion of embryo.

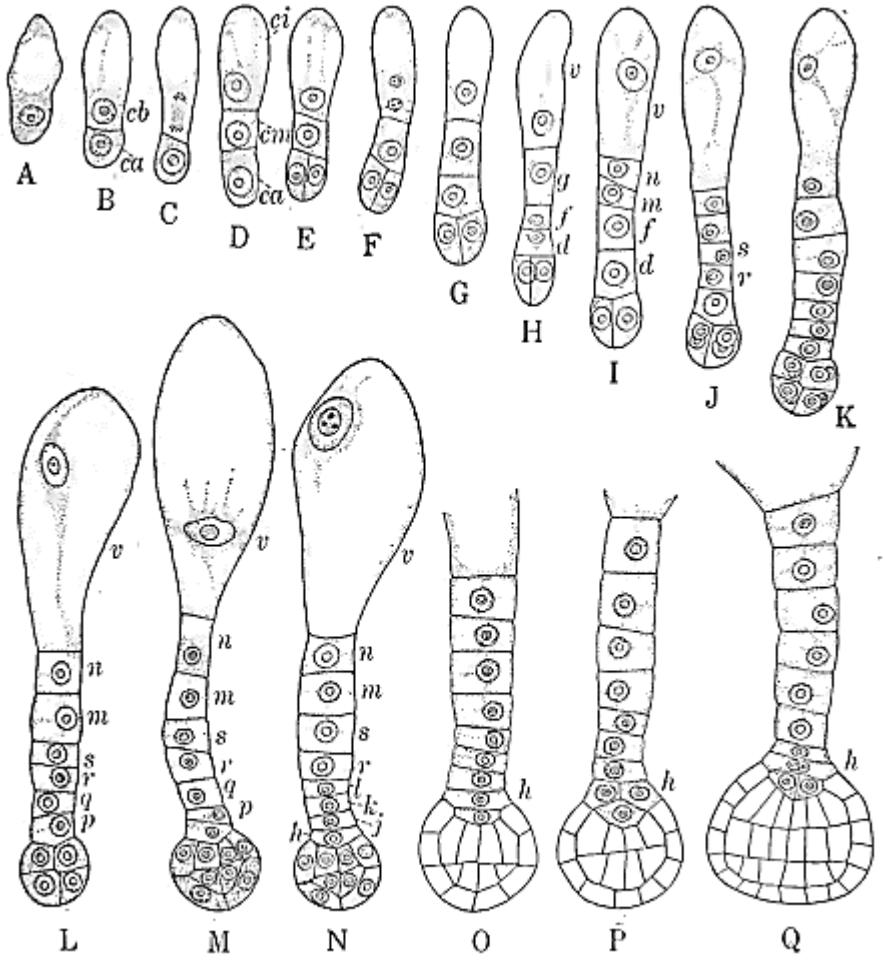


FIG. 145. Development of embryo in *Capsella bursa-pastoris*. (*After Soueges, 1914, 1919.*)

Formation of suspensor and radicle: The suspensor cell undergoes few transverse divisions. It produces short filament of cells called **suspensor.** The first cell of suspensor enlarges very much. It becomes basal cell. It pushes the embryo down into the developing endosperm. Suspensor also acts as conductive tissues for the nutrients. The last cell of suspensor adjacent to embryonal cell is called **hypophysis.** Hypophysis divides further to form **radicle.**

Formation of octant: They embryonal cell increases in size. It divides by three divisions. Two divisions are vertical and one division is transverse. These divisions form eight groups of cells called **octant or pro-embryo.** The four octants towards the chalazal end are the epibasal **or anterior octant.** The other four octants which are adjacent to suspensor are **hypobasal** or **posterior octant.**

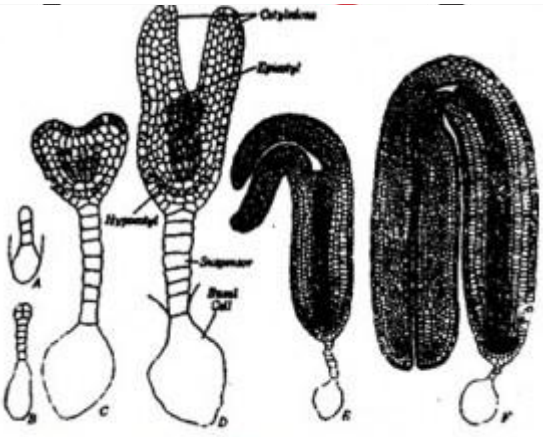


Fig. 'ages of development of Capsella bursa pastoris

Formation of cotyledons and plumule: The epibasal cells further divides to form two cotyledons and plumule. Further divisions occur in the cotyledonary cells and bilobed mass of cells is formed. These lobes are primary cotyledons. The plumule and epicotyl is produced in the notch between two depressions. Therefore, plumule in dicot is terminal in origin.

12. **Formation of hypocotyl:** The hypobasal octants divide to form mass of cells called **hypocotyl.** Hypocotyl is elongated. It carries radicle at its tip.

13. **Folding of embryo:** The developing embryo increase in size. Therefore, it become curved or folded in different ways. The way of folding of embryo in seed is characteristic feature of each plant.

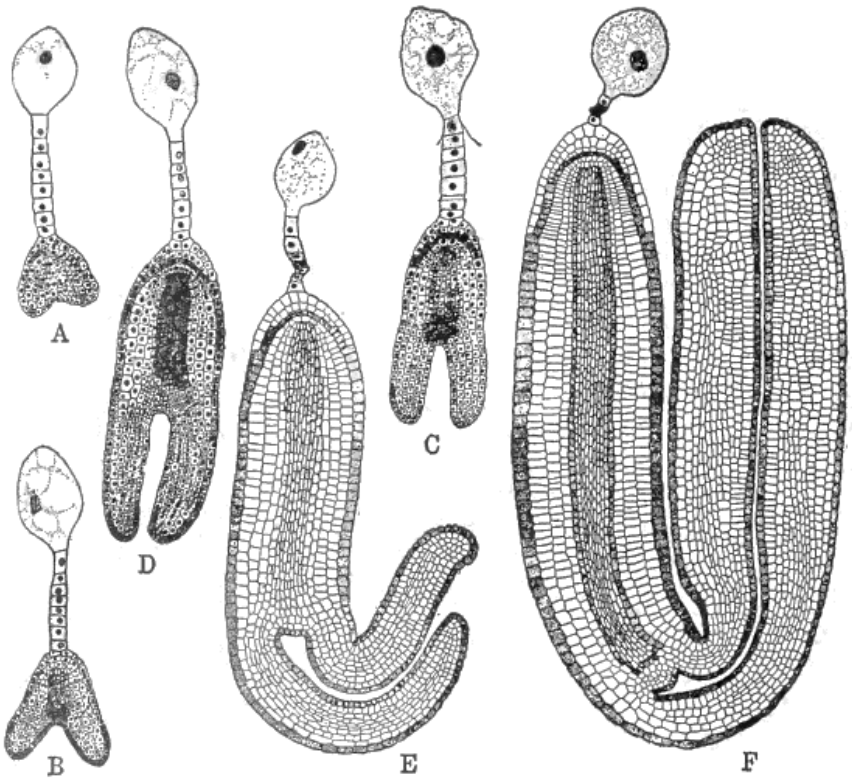


FIG. 146. Older stages in development of embryo of *Capsella bursa-pastoris*. (*After Schaffner, 1906.*)

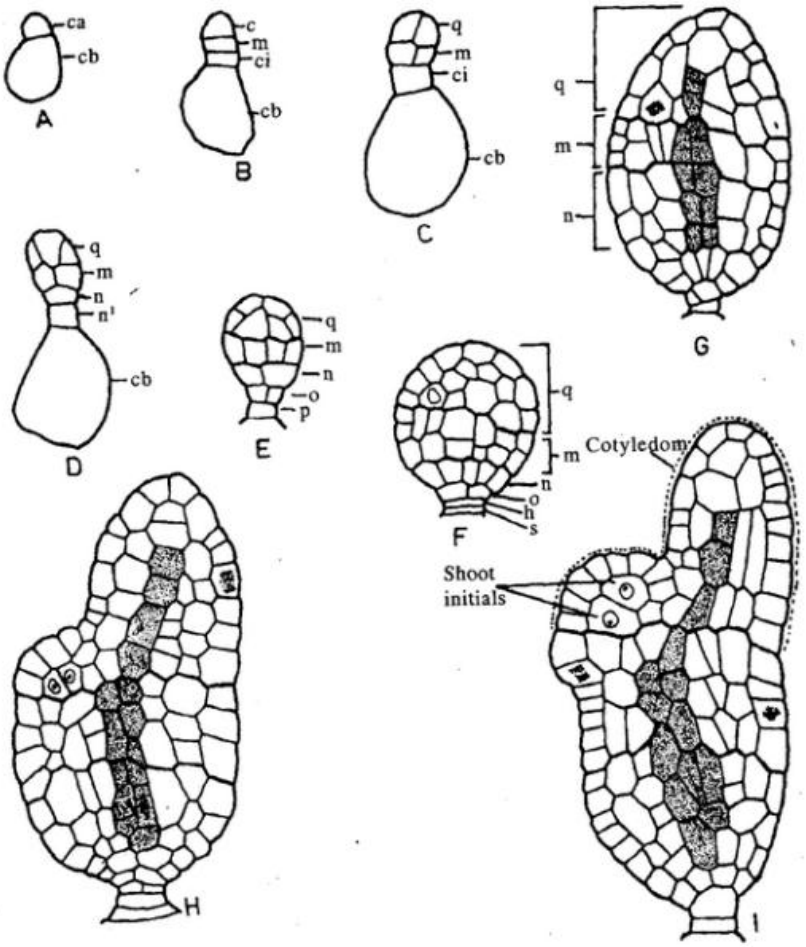


Fig. 11.7. Development of embryo in *Najas lacerata* (ater Swamy and Lakshmanan, 1962)

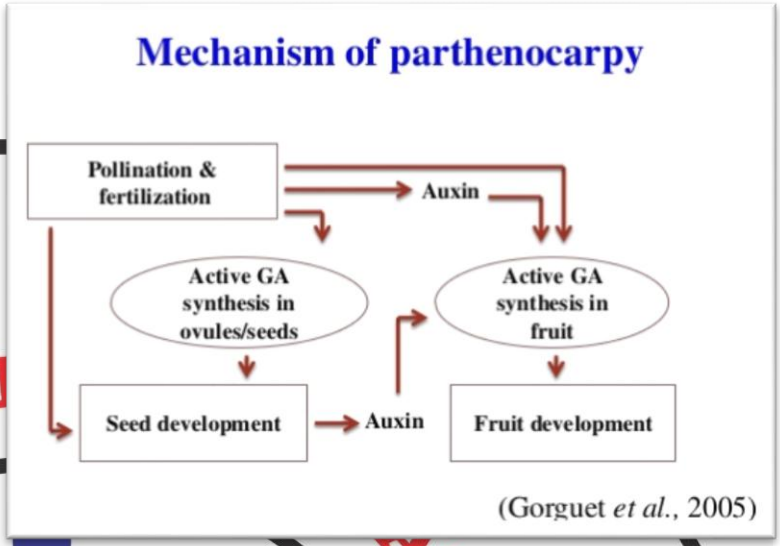
14. **Formation of basic layers of meristem:** Two successive divisions occur in octants. It produces three layers. The outer layer is called **dermatogen,** middle is called **periblem** and central one is called **plerome.** Dermatogen gives rise to epidermis. Periblem gives rise to cortical portion. Plerome forms the stele in the centre.

Embryogenesis in Monocot (*Najas lacerata)*

A typical monocot type of the embryo development can be seen in *Najas lacerata*. In this the first division of the zygote is transverse resulting in the formation of an apical cell *ca* and the basal cell *cb* (Fig. 11.7A). The basal cell *cb* does not divide further and transforms itself into a large vesicular structure, which forms a part of the suspensor (Fig. 11.7 A-D). Thus the entire embryo is derived from apical cell *ca*. During further development, the *ca* undergoes a transverse division and forms two cells namely *c* and *d.* Of these the cell *d* once again divides transversely. In this way a linear proembryo of four cells (*c, m, ci, cb*) is formed (Fig. 11.7 B). Two vertical divisions at right angles to each other in the two distal cells (*c* and *m*) lead to the formation of two superposed tiers (*q* and *m)* of four cells each (Fig. 11.7 C, D). In the meantime cell *ci* divides transversely to give rise to *n* and *n'* (Fig. 11.7 D). Whereas cell *n* divides vertically, *n'* undergoes transverse division giving rise to two cells (*o* and *p* Fig. 11.7E). During further growth (Fig. 11.7 F-I) tier *q* gives rise to single cotyledon, *m* gives rise to the hypocotyls. The radicle is organized from the derivatives of *n* while *o* gives rise to root cap. Derivatives of *p* form suspensor.

PARTHENOCARPY:

Development of fruits without fertilization is called parthenocarpy and the fruits thus produced are known as parthenocarpic fruits or seedless fruits, e.g. *Musa* (Banana), *Psidium* (Guava), *Pyrus* (Apple), *Vitis* (Grapes) etc. The term parthenocarpy was coined by Noll (1902).



Types of Parthenocarpy:

Nitsch (1963) has recognized three types of parthenocarpy:

(a) Genetic parthenocarpy: Several cultivating plants have both seeded as well as seedless fruits. These seedless fruits are formed parthenocarpically due to hybridization or mutation. The famous seedless navel variety of orange was developed from a normal seed-bearing variety of *Citrus* through mutation in axillary bud that grew out into a branch bearing seedless fruits.

(b) Environmental parthenocarpy:

Environmental conditions such as fog, frost, high temperature, freezing interfere with the normal functioning of reproductive organs and bring about parthenocarpy in plants e.g., Heavy fog during month of June helps formation of seedless fruits in olives, parthenocarpic fruits in *Capsicum* by keeping the plant at relatively low temperature (6° to 10°C) at the time of anthesis, in Pears by placing its flowers to freezing temperature for 3 to 19 hours.

(c) Chemically induced parthenocarpy: Thimann (1934) gave the idea that pollen grains have auxin and other growth regulatory substances that have stimulatory effects on the female sex organs. Artificial application of 0.5 to 1% Solution of IAA (Indole Acetic Acid), α-Naphthalene acetic acid, para chlorophenoxy acetic acid, phenyl acetic acid, gibberellins etc.

Significance of Parthenocarpy:

1. Parthenocarpic fruits have an increased proportion of edible part than in normal fruits.
2. In horticulture seedless fruits are suitable either as consumption or in the preparation of jams and juices.

POLYEMBRYONY:

Meaning of Polyembryony:

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

In plants, this phenomenon was first reported by Antoni van Leeuwenhoek (1719) in orange seeds. In several gymnosperms, the polyembryony is so common that it might be regarded as an important character of this group. In majority of the gymnosperms showing polyembryony, usually two or more archegonia develop in a female gametophyte. And as each archegonium contains an egg, two or more eggs may be fertilized and thus two or more potential embryos may be created. Only one embryo, however, survives usually, and all the others perish during the course of the development.

Polyembryony can be broadly categorized into two groups.

1. True polyembryony: Many embryos are developed inside single embryo sac.
2. False polyembryony: If the ovule carries more than one embryo sac & embryos develop in each embryo sac.

Polyembryony may be of following two types:

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

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

Yakovler (1967) has distinguished two types of spontaneous polyembryony:

(a) Gametophytic:

Arising from gametic cell of embryo sac.

(b) Sporophytic:

Arising from zygote, proembryo or initial sporophytic cells of the ovule (nucellus, integuments). Embryo development can also be made in culture medium (induced polyembryony). The embryos developed in culture medium are known as adventitious embryos, somatic embryos, supernumerary embryos or embryoids.

True Polyembryony takes place in angiosperm due to:

1. Cleavage of proembryo:
2. Development of many embryo from synergid, antipodal cells, endosperm except egg.
3. Development of many embryo due to presence of more than one embryo sac.
4. Development of polyembryo from nucellus, integument (outside the embryo sac).

1. Cleavage polyembryony:

It results from the cleavage of the zygote or earlier stages of its development (proembryo) into two or more units e.g. *Nicotiana rustica*, *Isotoma longiflora*, *Lobelia*, *Erythronium*. Cleavage polyembryony is common in gymnosperms, but it is of rare occurrence in angiosperms.

In *Erythronium americanum*, first division of the zygote is normal. From embryonic mass, many cells at distal end form separate embryos (Fig. 2.35).

In *Isotoma* and *Exocarpus*, additional embryos are formed from suspensor cells of proembryo.

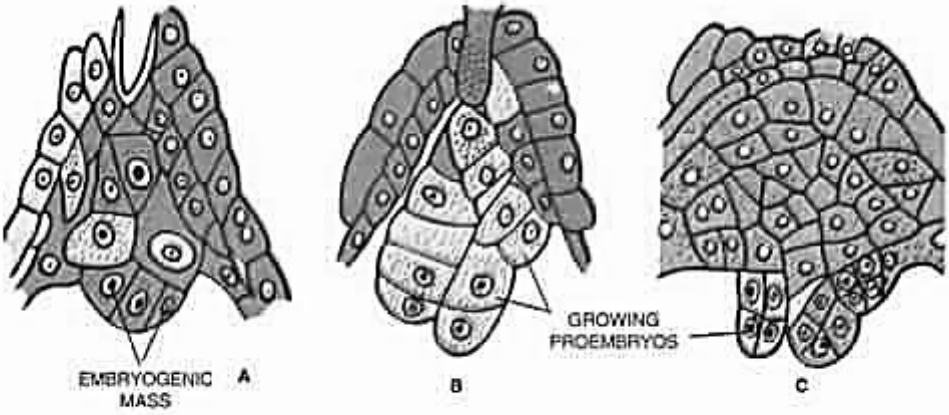


Fig. 2.35. A-C. Cleavage polyembryony : **A.** Embryonic mass formed by the basal cell of the zygote in *Erythronium americanum*, **B-C.** Differentiation of embryos from the cells of the embryonic mass.

2. Embryos from cells of embryo sac other than egg:

The embryo may appear from synergids and antipodal cells in the embryo sac. The synergids may be fertilized by sperms from an additional pollen tube or develop without such fusion. In *Argemone mexicana* and *Phaseolus vulgaris*, additional embryos may appear from unfertilized synergids and hence haploid in nature. Embryos from

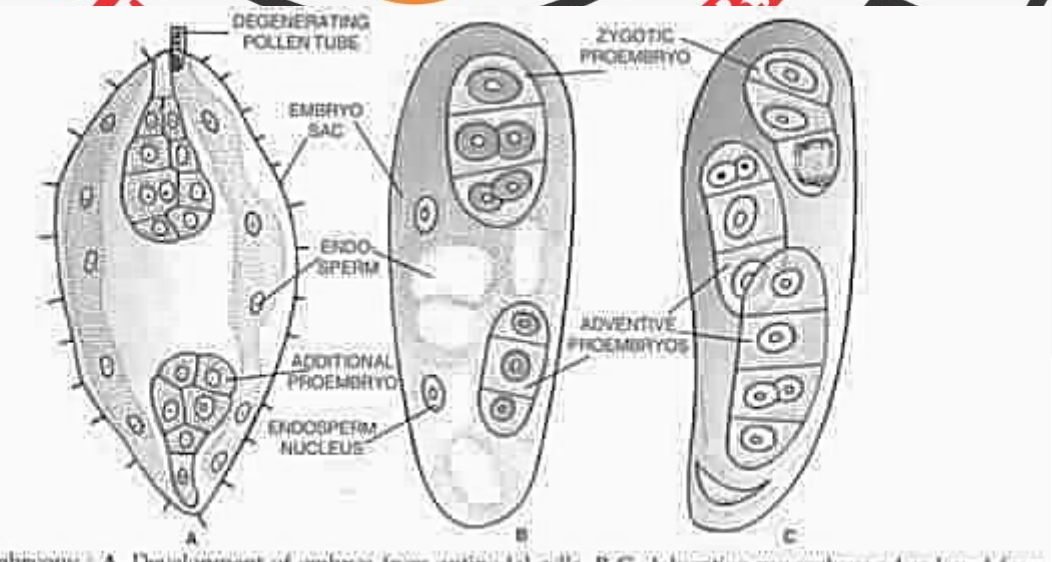


Fig. 2.36. Polyembryony : **A.** Development of embryo from antipodal cells. **B-C.** Adventive pro-embryos developed from the cells of nucellus (they grow along with the zygotic embryos).

antipodal cells (Fig. 2.36) develop less frequently (e.g. *Ulmus Americana*, *Allium odorum*). All the antipodal embryos may not remain viable.

***3.* Embryos from endosperm:**

Embryos developing from endosperm have been reported in *Balanophora* (Treu, 1898), *Alnus* (Woodworth, 1930). However, Ernst (1913) found that such embryos develop from egg, got embedded in cellular endosperm.

4. Embryos arising from the cells outside embryo sac:

Cells of the nucellus and integuments have also been observed to develop into embryos e.g. *Citrus*, *Eugenia* and *Mangifera*. In *Spiranthes*, additional embryos have been reported to be developing from inner cells of inner layer of integument (Swamy, 1948). Such embryos subsequently come to lie in the embryo sac and are nourished by the endosperm. According to Haberlandt (1921)" Stimulus for polyembryony is provided by degenerating cells of nucellus. (Necrohormone theory)

Practical value of polyembryony:

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

APOMIXIS:

Sexual reproduction (Amphimixis) normally carries two regular features i.e Meiosis & Fertilisation. But in some plants abnormal kind of amphimixis takes place in which egg or cell in embryo sac (synergid, antipodal cell) develop into an embryo without fertilisation and with or without meiosis. "Apomixis is an abnormal type of sexual reproduction where there is no meiosis & fertilisation."

Types of Apomixis:

It is mainly of two types:

1. Vegetative reproduction:

New plants develop from parts other than seed.

2. Agamospermy:

In this case embryo produced inside seed by abnormal process. It is two types

(a) Adventive Embryony:

Development of embryo directly from sporophytic tissue (2n) i.e, nucellus, integuments.

(b) Diplospory:

Megaspore mother cell without meiosis develops into diploid embryo sac. Though diploid egg develop embryo without fertilisation. It is also known as diploid Parthenogenesis.

(c) Apospory:

Development of embryo sac directly from cell of nucellus. According to Panchanani Maheshwari; Apomixis maybe of two types.

1. Recurrent Apomixis: it consists of Vegetative propagation & agamospermy.
2. Non-Recurrent Apomixis:

It is of two types:

(a) Haploid Apogamy: Development of embryo from cells inside the embryo sac other than egg.

(b) Parthenogenesis: Development of embryo from egg without fertilisation.

SEED – STRUCTURE OF DICOT AND MONOCOT SEED

A true seed is defined as a fertilized mature ovule that possesses embryonic plant, stored food material, and a protective coat or coats. It serves the function of perennation, dispersal and reproduction of the parent plant. In angiosperms, the seeds are enclosed within fruits. The structure of seeds may be studied in such common types of pea, gram, bean almond or sunflower. They are all built on the same plan although there may be differences in the shape or size of the seed with the relative proportion of various parts.

There are hundreds of variations in the seed size, shape, colour and surface. The seeds range in size from tiny dust particles, as found in some orchids, to large double-coconuts. The seed surface may be smooth, wrinkled, striate, ribbed, furrowed, reticulate, tuberculate, alveolate, hairy, and pulpy or having patterns like finger prints.
In the seed, life activities are temporarily suspended in order to enable the plant to successfully pass through unfavourable and injurious climatic conditions. On the approach of favourable conditions, the seed resumes active life and grows into full plant. In the form of seeds, a plant can be carried to long distances without special precautions.

General Structure of a Seed:
A seed is generally made up of seed coat and embryo.

(a) Seed Coat:
It is the protective covering of the seed derived from one or both integuments of the ovule. Usually seed coat is two layered. The outer thick and hard layer is called testa, while the thin inner membranous layer is called tegmen. The surface of the seed possesses a fine pore at one end. It is called micropyle. There is also a scar called hilum. It is the place where funiculus or stalk of the seed is borne. Some seeds also show chalaza (place of origin of seed coats) and raphe (part of funiculus fused with seed wall).

(b) Embryo:
It represents the dormant future plant that remains enclosed within the seed coat. The embryo consists of an axis or tigellum to which are attached one (in monocotyledonous seeds) or two (in dicotyledonous seeds) seed leaves or cotyledons.
The embryo axis consists of plumule, epicotyl, cotyledonary node, hypocotyle and radicle. Plumule represents the embryonic shoot while radicle represents embryonic root. The embryo axis has a node called cotyledonary node that bears one or two cotyledons (= seed leaves).

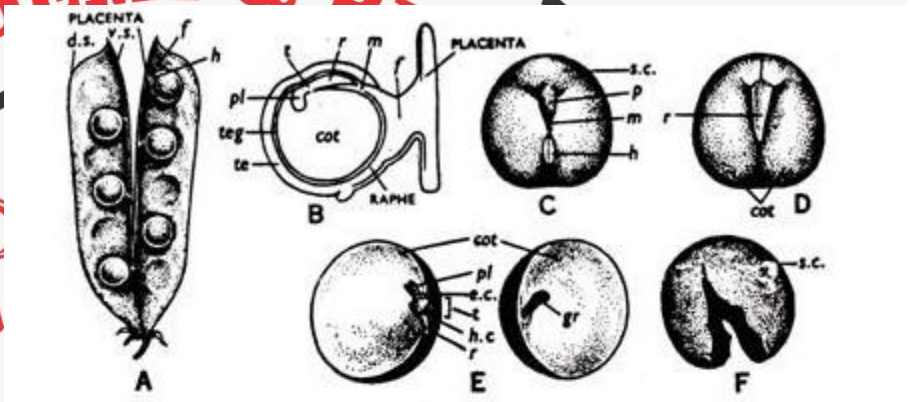


Fig. 30

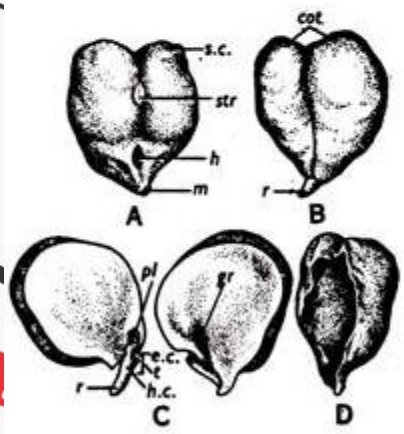


Fig. 31

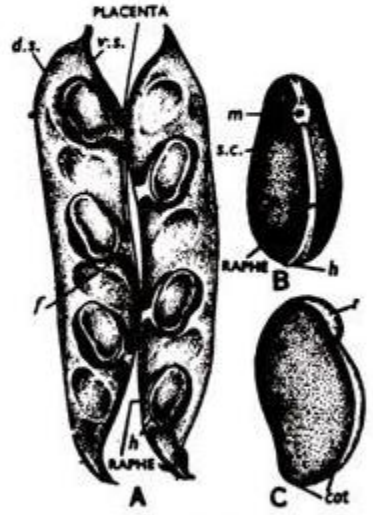


Fig. 32

Dicotyledonous Exalbuminous Seeds. Fig. 30. The pea seed. A. Pea pod opened to show positions of seeds. B. Diagrammatic section through seed and placenta. C. Whole seed. D. The kernel after removal of seedcoat. E. Kernel with cotyledons dissected. F. Removed seedcoat. Fig. 31. Gram seed. A. Whole seed. B. The kernel after removal of seedcoat. C. The two dissected cotyledons. D. Peeled seedcoat. Fig. 32. Lablab Bean (*Dolichos lablab*) seed. A. Open pod. B. Whole seed with prominent raphe. C. Kernel after removal of seedcoat. *d.s.*=dorsal suture; *v.s.*=ventral suture; *f*=funicle; *h*=hilum; *te*=testa; *teg*=tegmen; *m*=micropyle; *cot*=cotyledon; *t*=tigellum; *pl*=plumule; *r*=radicle; *s.c.*=seedcoat; *p*=pouch containing radicle; *e.c.*=epicotyl; *h.c.*=hypocotyl; *str*=strophiole; *gr*=groove for plumule

The part of embryo axis between plumule and cotyledonary node is called epicotyle and a similar region between cotyledonary node and radicle is called hypocotyle. The seed contains reserve food either in cotyledons or in a special tissue called endosperms.

Type 1. Dicotyledonous Exalbuminous Seeds:

A typical example is common pea (*Pisum sativum*). On carefully opening a mature green pod along the dorsal suture the placental tissue is seen to spread along the ventral suture and the roundish seeds are seen arranged in two rows along the length of the pod.

Each seed is attached to the placental tissue on the fruit suture by a stalk called the funicle. The funicle is narrow at the placental end but widens into a disc where it joins the seed. When the mature seed is detached, funicle leaves a scar on the seed called the hilum. Next to the hilum is a pinhole opening on the seed coat which is the micropyle. If the seed is soaked, wiped and then squeezed, water is seen to ooze out of this micropyle. The seed is covered by the tough seed coat (testa) of a light colour.

The tegmen, which is delicate and completely adherent to the inner side of the testa, is not distinguishable in the mature seed. On opening the seed coat the kernel (containing embryo) is obtained. In it the two fleshy cotyledons (stores all the nutrients required by the growing seedling) are very conspicuous.

The two cotyledons are hinged to an axis (tigellum) so that they open out like a book. The tigellum represents the axis of the future plant. One end of the tigellum is pointed and protrudes out of the cotyledons.

This lies next to the micropyle and is the radicle or the rudimentary root. The protruding radicle lies under the pouch-like expansion of the seed coat and is thus visible even when the seed coat is not removed.

The other end of the tigellum is the feathery plumule end which is the first apical bud of the future plant and develops into the shoot. The plumule lies in a groove inside the cotyledons. The point of attachment of the cotyledons to the tigellum is the first node on the axis and careful observation shows the presence of the first lateral buds in the axils of the cotyledons.

The portion of the tigellum just below the cotyledonary node (i.e., between radicle and node) is called the hypocotyl and the portion just above (i.e., between node and plumule) the node is the epicotyl.

All the dicotyledonous exalbuminous seeds conform to the above plan, though there may be /variations in details. In gram (*Cicer arietinum*), the seed is broad at one end and somewhat pointed at the other. On the seed coat, below the hilum, there is another more prominent scar, the strophiole, which is a scar left by a funicular outgrowth. The brown seed coat is the testa but on its inner side a papery white membranous layer may be distinguished as the tegmen. The embryo does not differ from that of pea except in shape.

There are various types of bean seeds of which *Dolichos lablab* is very common. The seeds occur in the pod as in peas. They are larger and more or less oval. The seed coat (mainly testa with a thin fused tegmen) is very hard and black, brown or red in colour. The funicle is extended into a long raphe which is seen above the hilum. The embryo is as usual.

Type 2. Monocotyledons, with only one cotyledon. Ex: - Maize Grain (Fig. 5.136):

External Structure (Fig. 5.136 A):

Maize grain is a single-seeded fruit may be whitish, yellow, violet or red in colour. It has a smooth and shining surface. The grain is conical and flattened. It is attached to the cob by its narrow-pointed end which is surrounded loosely by a shallow husk.

The broader end is roundish. Near the broader end the upper flat surface contains a small papilla which represents the remains of the style. On one side of the grain a small, opaque, whitish, deltoid area is seen to be distinctly marked out from the region. The embryo lies embedded in this area.

Internal Structure (Fig. 5.136 B):

On the outside of the grain is present a single thin but hard covering. It is formed by the fusion of the seed coat or testa and the fruit wall or pericarp.

Below the grain covering are present two structures, endosperm and

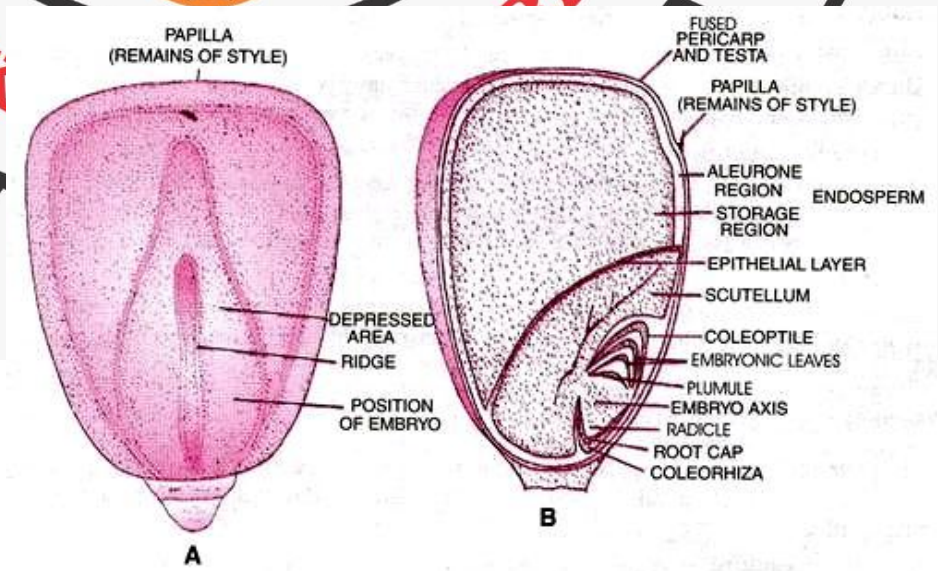


Fig. 5.136. Structure of Maize grain. A, external structure. B, L.S. or V.S. grain.

embryo. The endosperm occupies most of the interior of the grain on the broader and the lower sides. It consists of two parts, horny aleurone and mealy storage.

The aleurone region lies immediately below the grain covering. It is 1-3 celled in thickness. The cells have thick walls and dense cytoplasm filled with aleurone or protein or protein grains. The latter produce enzymes during the process of grain germination.

The storage region of endosperm is whitish or yellowish. It has large thin walled cells with disintegrated cytoplasm and rich in starch grains. The cells also possess fats and proteins. The embryo occurs in the pointed part of the grain, mostly towards the upper side.

It consists of an embryo axis containing a radicle, a plumule and a single lateral cotyledon. The radicle (or future first root) lies at pointed end of the grain. It has two protective sheaths, inner root cap and outer coleorhiza. The plumule (or future shoot) lies towards the broader side of the grain at the other end of embryo axis.

It bears a few rudimentary leaves and a conical protective sheath known as coleoptile. Coleoptile has a terminal pore for the emergence of first leaf during germination. The sheath is capable of growth. It assists the future shoot in passing through the soil during germination.

The single cotyledon of Maize grain is called scutellum. It occupies the major portion of the embryo region of the grain. The outermost layer of scutellum lying at the boundary of endosperm and embryo is known as epithelial layer. It is both secretory and absorptive.

The epithelial layer secretes hormones into the endosperm for the synthesis of enzymes required for solubilisation of food. The solubilized food is absorbed by it and then transferred to the embryo axis. Roughly in the middle of embryo axis arises a vascular strand. It ramifies into the scutellum. The place of origin of the vascular strand from the embryo axis is called cotyledonary node.

FIG. 40

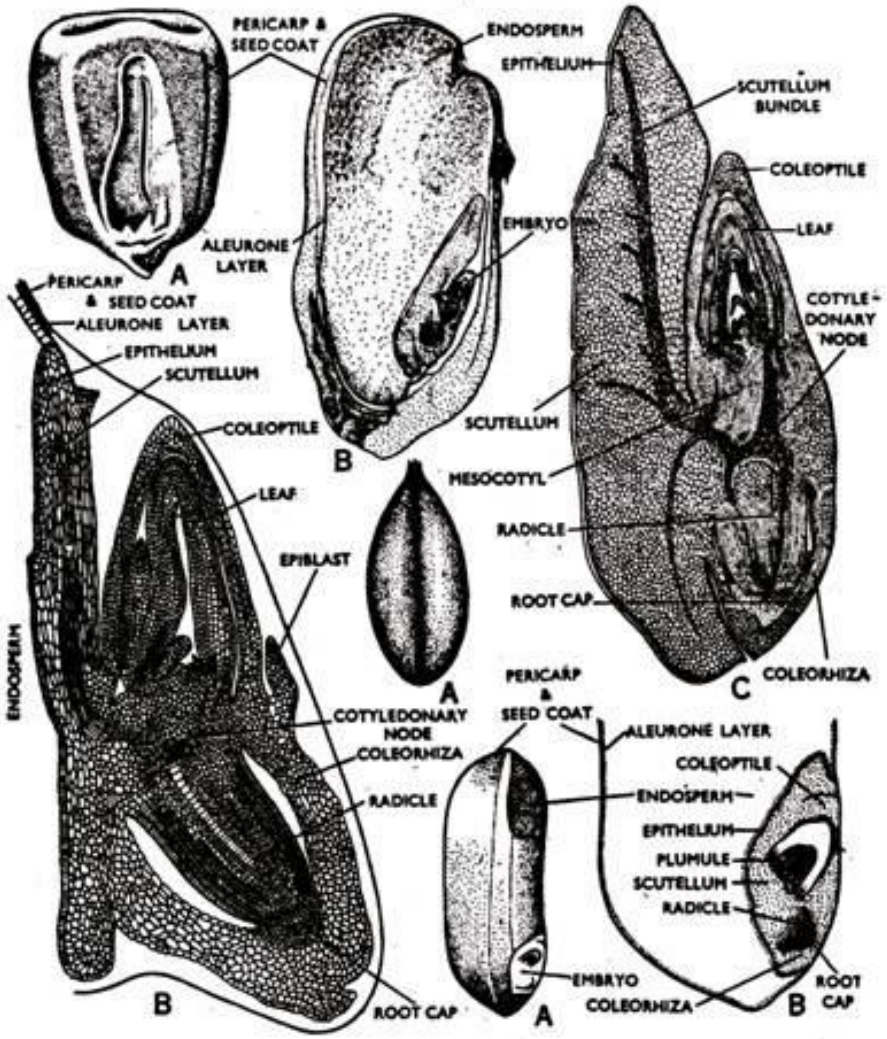


FIG. 41 FIG. 42

MONOCOTYLEDONOUS ALBUMINOUS SEEDS. FIG. 40. Seed (fruit) of Maize. A. Whole grain. Note the triangular position of embryo. B. Longitudinal section. C. Embryo in l.s. FIG. 41. Seed (fruit) of Wheat. A. Whole grain. B. Embryo showing a part of endosperm in l.s. FIG. 42. Seed (fruit) of Rice. A. A grain of rice showing sectional view of a bit of endosperm and embryo. B. Lower portion of A magnified.

PALYNOLOGY

POLLEN MORPHOLOGY

The science concerning the study of pollen and spores is called 'palynology", and this term was coined by Hyde and Williams in 1945.

"Pollen grains" or microspores are the male reproductive bodies of the flowering plants, while the term "spore" is very loosely applied to several types of reproductive bodies in algae (e.g. zoospores, exospores, endospores, akinetes, etc.), fungi (e.g. conidiospores, ascospores, uredospores, basidiospores, chlamydospores, etc.) and pteridophytes. Pollen grains develop in the sporogenous tissue of anthers or microsporangia in angiosperms.

According to Zetzsche and Vicuri (1931) the outer walls of pollen and spores are made up of a pectinous substance called "pollenin". Its chemical formula is $C_{90}H_{129}(OH)_5$. The protoplasm of pollen grains contains proteins, carbohydrates, lipids, vitamins, hormones and enzymes. It also contains traces of some inorganic substances such as Mg, K, Ca, Cu, Fe, Si, P, S and CI.

POLLEN ARCHITECTURE

CHARACTERISTIC # 1. POLLEN UNITS:

The pollen grains are produced within the anther of the flower. Pollen mother cells originate from the sporogenous tissue of the anther which later divide meiotically to form four pollen grains called tetrad.

The pollen grains do not remain united at maturity, and are dissociated into single pollen grain called monad. Sometimes rarer types like dyads (two pollen grains), Octads (eight pollen grains) and Polyads (many pollen grains) are also observed (Fig. 4.1).

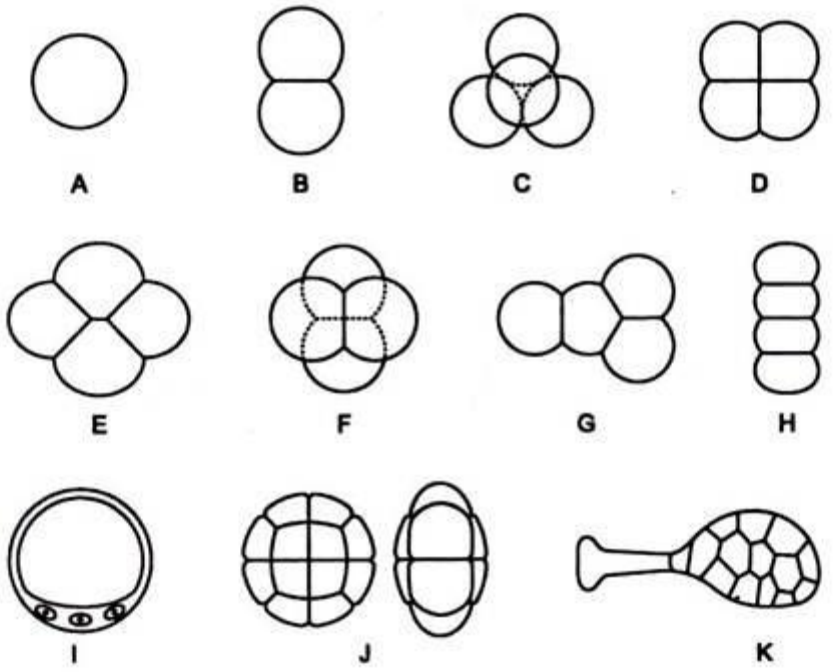


Fig. 4.1 : Pollen units (A = Monad, B = Dyads, C = Tetrahedral tetrad, D = Tetragonal tetrad, E = Rhomboidal tetrad, F = Decussate tetrad, G = T-Shaped tetrad, H = Linear tetrad, I = Cryptotetrad, J = Polyads, K = Pollinia)

CHARACTERISTIC # 2. POLARITY:

Some Common Terms:

The orientation of polarity is an important criterion in identification and description of pollen grains, as apertural position is of primary phylogenetic and functional significance. All pollen grains are in tetrad stage during development and the polarity is determined in this stage, prior to their separation.

The part of the pollen grains which is nearest to the centre of the tetrad is the proximal pole and that towards the opposite side is the distal pole (Fig. 4.2). The imaginary line between the proximal and distal pole of the grain is called the Polar Axis (PA) which passes through the centre of the spore to the centre of the tetrad.

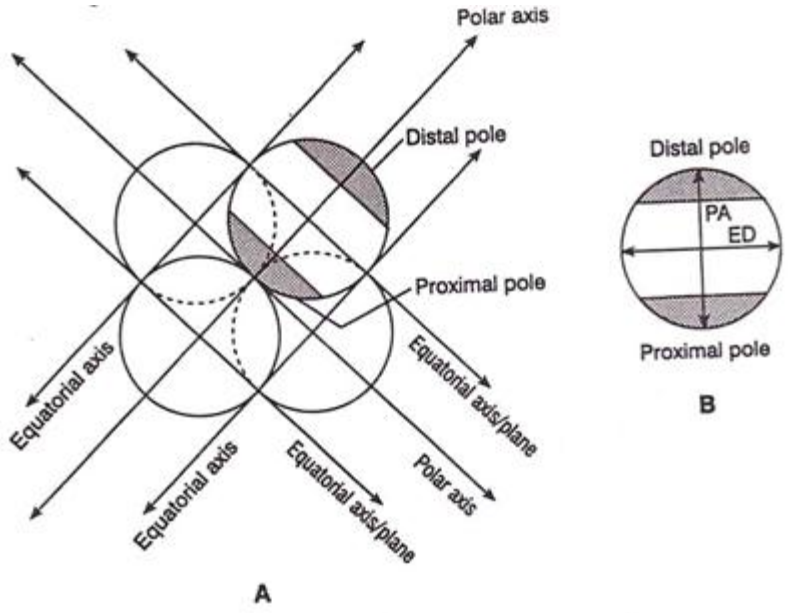


Fig. 4.2 : Polarity (A = Showing polarity in terad stage; B = Showing the length of polar axis (PA) and breadth of equatorial diameter (ED) in a monad grain)

The plane perpendicular to the polar axis through the middle of the grain is the equatorial plane (equatorial diameter). Positions on the surface of the grain maybe determined by their latitude, comparing to the latitude on a regular sphere. Similarly, surface features in a pole to pole direction at right angles to the equatorial plane are called meridional.

The pollen grains maybe either apolar or polar.

In apolar spores, poles or polar regions cannot be distinguished in individual spore (monad) after separation from tetrad. Among the polar types the pollen grains are either isopolar or heteropolar depending upon the demarcation between two equal or unequal polar faces, respectively (Fig. 4.3).

In isopolar grains the distal and proximal faces (above and below the equatorial plane) look alike.

In heteropolar grains the two faces are distinctly different, either in shape, ornamentation or apertural system. Thus one face may have an opening (aperture) and the other not.

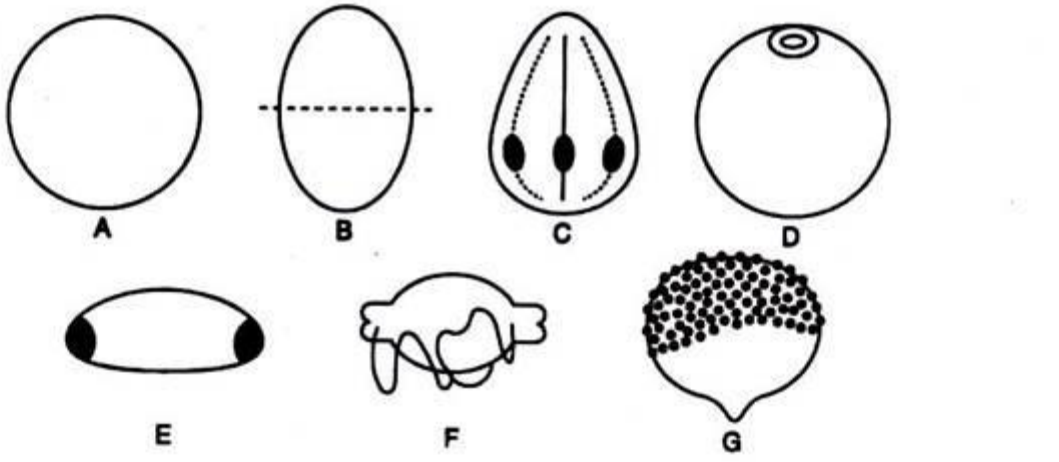


Fig. 4.3 : Polarity (A = Apolar; B = Isopolar, C&D = Heteropolar, E&F = Paraisopolar, G = Cryptopolar)

The pollen grains showing slight differences between the distal and proximal faces are also called paraisopolar or subisopolar (Fig. 4.3). Say for example, one face (distal) is convex and the other face (proximal) is plane or concave or vice versa. Their equatorial plane is usually more or less curved. Sometimes there are small differences in the surface details of the two poles viz. Carya, Ulmus, etc.

In some bryophyte spores like Calobryum dentatum, Haplomitrium hookeri, the distal and proximal faces have dissimilar sculpturing and lack tetrad mark. This type of spores is called Cryptopolar (Fig.4.3).

CHARACTERISTIC # 3. SYMMETRY:

Pollen grains or spores are symmetric or asymmetric.

The asymmetric grains are either non- fixiform (without fixed shape) or fixiform (with fixed shape). Asymmetrical grains have no plane of symmetry. They are rare in occurrence.

The Symmetric grains are either radiosymmetric (radially symmetrical) or bilateral (having a single plane of symmetry) (Fig. 4.4).

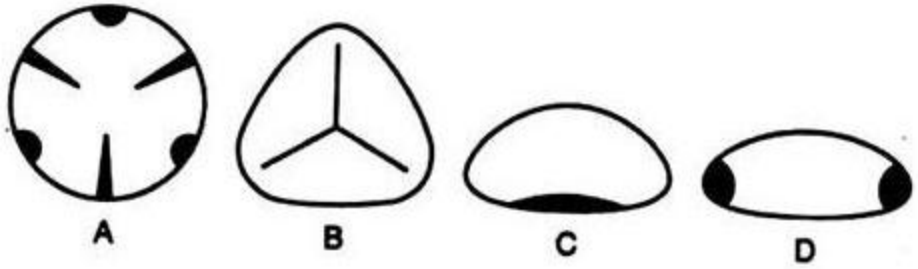


Fig. 4.4 : Symmetry (A & B = Radially symmetric, C & D = Bilateral)

In radiosymmetric grain the shape is such that any plane including the polar axis that passes through will produce identical halves. So, the radiosymmetric grains have more than two vertical planes of symmetry. Radially symmetrical isopolar grains have one horizontal and two or more vertical planes of symmetry. Radially symmetrical heteropolar grains have no horizontal plane of symmetry.

Bilateral heteropolar pollen grains have two vertical planes of symmetry. Bilateral isopolar grains have three planes of symmetry, one horizontal and two verticals. In some bean- shaped or boat-shaped spore/pollen there is only one vertical plane of symmetry with an opening towards the end of the grain.

CHARACTERISTIC # 4. POLLEN WALL

Intine and Exine:

The protoplasm of the pollen grains is enclosed by a wall made of intine and exine. The intine is a hyaline layer. The exine (Fig. 6) consists of an inner homogeneous layer (called endine or nexine and an outer heterogeneous layer (called ectine or sexine).

Ectine and Endine:

Ectine is the outer layer of exine while endine is the inner layer of exine

Columellae and Tegillum:

The radial rods which form the ectine are called columellae. The columellae are either free at their tips or are fused to form a layer called tegillum.

Exine Ornamentation:

Various types of ornamentation patterns are shown by exine surface. As mentioned above, the ectine of exine is composed of radial rods or columellae. When the distal surfaces of columellae are bright and the intervening regions are dark, the pattern is called pilate (Fig. 6). The columellae in most of the grains are fused to form different types of patterns having depressed areas (called lumina) and the intervening areas between lumina (called muri).

When a network is produced by lumina and muri it is called reticulate. In the reticulate pattern, if there is the incomplete fusion of columellae, it is called retipilate, if there are circular and closely placed lumina it is called foveolate, if there are circular but distantly placed lumina it is called scrobiculate, if lumina are elongated it is called fossulate, if lumina are parallel it is called striate and; if lumina are anastomosing it is called rugulate.

The exine ornamentation is called areolate when luminoid network surrounds islands of raised areas. If the excrescences (outgrowths) on the exine are in the form of very minute granules the pattern is called granulose, if the excrescences are in the form of spinules with pointed or blunt ends it is called spinulose, if the excrescences are in the form of rounded warts with constricted base it is called gemmate, and if the bases are not constricted it is called verrucate.

When the outgrowths on the exine are in the form of tubercles it is called tuberculate, if these are long with pointed ends the pattern is called spinose, if outgrowths are rod shaped it is called baculate, and when they are club shaped it is called clavate.

LO- or OL- Pattern:

In LO or OL pattern 'L' (lux) stands for "light" and O (obscuritas) stands for darkness. At different foci, a varying pattern of bright and dark islands is observed. At the uppermost focus, the depressed ends on the surface of pollen grains appear dark, and this darkness changes into brightness when focused down.

OLO- Pattern:

A succession of three patterns, i.e., dark (O), bright a) and dark (O) is called OLO-pattern.

CHARACTERISTIC # 5. POLLEN APERTURE

Morphologically aperture is an opening or thinning of the exine where the intine is usually thick; physiologically it is a germination zone or a harmomegathus (A mechanism accommodating changes in volume of the semirigid pollen exine) or both.

With regard to their position the apertures are polar, global or equatorial. The polar apertures are either monopolar (either in proximal or in distal pole) or bipolar (both in proximal and distal face). Global apertures are uniformly distributed over the pollen/spore surface. Equatorial apertures are meridionally arranged.

Some taxa have 'atreme' (trema, a Greek word means aperture) pollen/spore, i.e., they seem to have no special aperture, are termed as 'inaperturate' or non-aperturate.

Majority of the pollen grains described as 'inaperturate' seem to be 'omniaperturate', that is, the entire pollen wall is made up of a thin exine and a thick intine or at least thick as the exine, for example *Canna* sp. of Cannaceae.

There are two types of apertures known as Pores (Porus, p1. Pori) and furrows (Colpus, p1. Colpi. or Sulcus, p1. Sulci). In most cases the furrows act as harmomegathi.

Colpi and Colpate: The elongated or furrow-like apertures on the pollen grains are called colpi (sing., colpus) and such grains are called colpate (Fig. 5).

Pori and Porate: The circular apertures on the pollen grains are called pori and such grains are called porate (Fig. 5).

In colpi and pori both, the outer face of the aperture is congruent with the inner face.

Colporate: When the outer and inner faces of apertures are incongruent (Fig. 5), the apertures are called colporate.

(In most of the gymnosperms and monocots the apertures are known to be distal while in pteridophytes they are proximal).

Zonocolpate and Zonoporate: When the colpi or pori are zonal in position, they are called zonocolpate or zonoporate, respectively.

Pantocolpate and Pantoporate: When the colpi or pori are uniformly distributed on the exine surface, they are called pantocolpate or pantoporate, respectively.

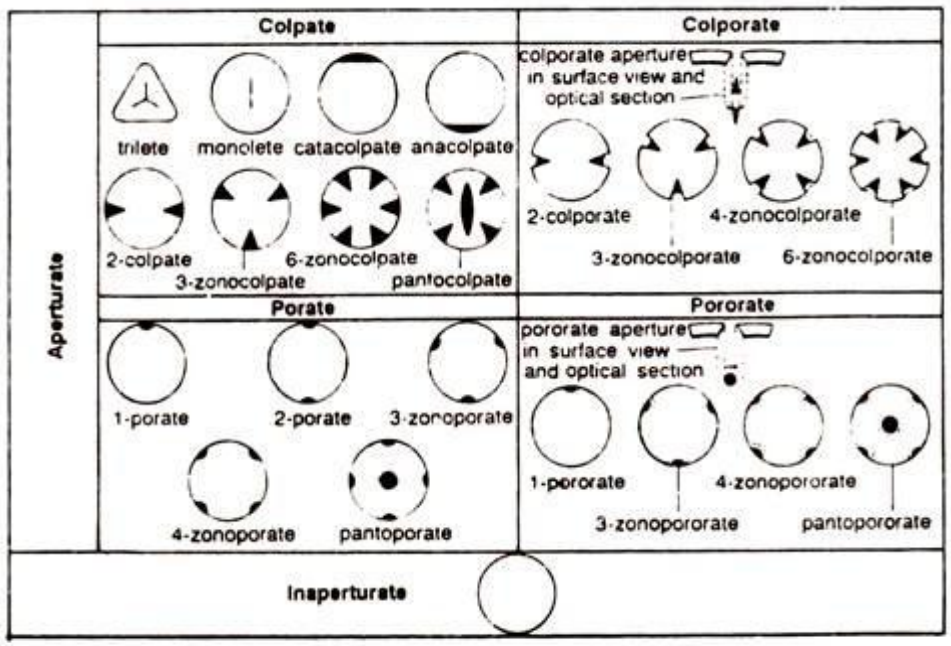


Fig. 5. *Types of apertures found in pollen and spores.*

Inaperturate: A pollen grain without any aperture (Fig. 5).

Crassimarginate: The apertures (colpi or pori) with thickened margins are called crassimarginate.

Syncolpate: When ends of the colpi unite at the poles, such grains are called syncolpate.

19. Ectocolpium and Endocolpium:

The outer and inner faces of a colpus are called ectocolpus and endocolpus, respectively.

20. Tenuimarginate:

The apertures (colpi or pores) with thin margins are called tenuimarginate.

CHARACTERISTIC # 6. POLLEN SHAPE:

The shape of the pollen grains varies from species to species. Shape of the grains is found to be useful in spore/pollen identification. However, the shape may vary considerably within one grain type or even within one species.

Pollen grains and spores are often described by the shape (non-angular and angular) of their outline both in polar and equatorial views. The shape of the pollen/spores may be circular, elliptical, triangular, rectangular, quadrangular or in other geometrical shapes (Fig. 4.5).

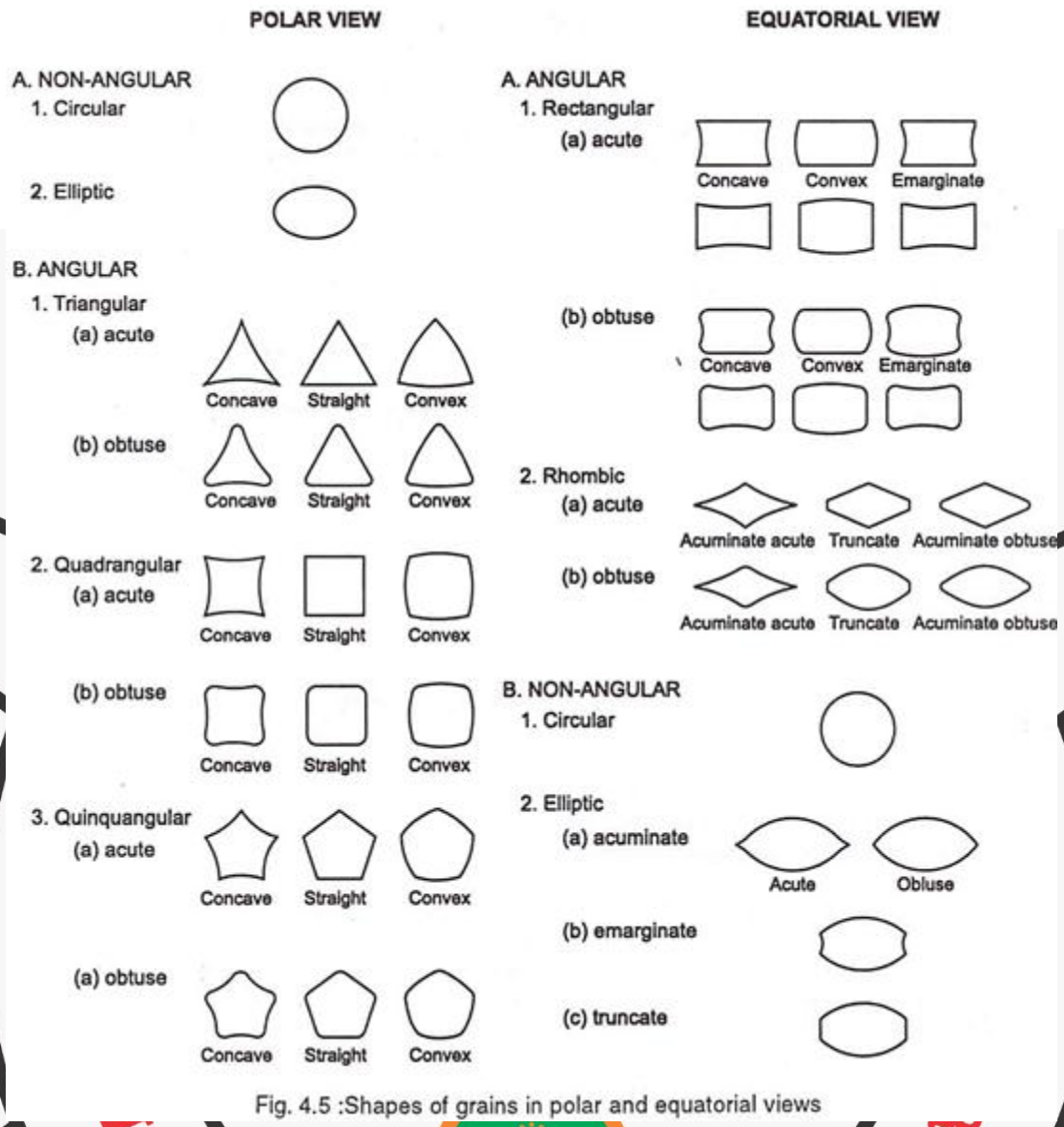


Fig. 4.5 :Shapes of grains in polar and equatorial views

G. Erdtman (1952) categorized eight shape classes based on the ratio of polar axis (PA) and equatorial diameter (ED). In the equatorial view, the ratio between the PA and ED, multiplying by 100 gives the indication of the shape.

Various PA/ED ratios are divided in it to different shape classes, e.g., Prolate, Prolate-spheroidal, Spheroidal, Sub-prolate, Perprolate, Oblate, Oblate-spheroidal, Sub-oblate, Peroblate (Table 4.1 & Fig. 4.6):

In bilateral grains, pollen are plano-convex, concavo-convex or biconvex in lateral view.

Table 4.1: Pollen shape classes (*after* Erdtman, 1952).

Shape classes	(PA/ED) × 100
Per-oblate	<50
Oblate	50-75
Sub-oblate	75-88
Oblate-spheroidal	88-99
Spheroidal	100
Prolate-Spheroidal	101-114
Sub-prolate	114-133
Prolate	133-200
Per-prolate	>200

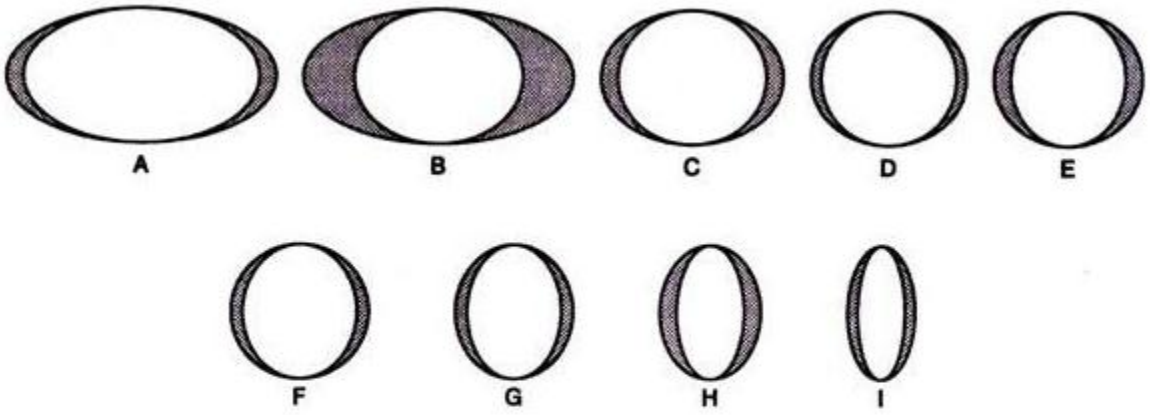


Fig. 4.6 : Shape classes (A = Peroblate, B = Obtate, C = Sub-oblate, D = Oblate-spheroidal, E = Spheroidal, F = Prolate-spheroidal, G = Sub-prolate, H = Prolate, I = Perprolate)

CHARACTERISTIC # 7 POLLEN SIZE:

Pollen grains show a great variety in their sizes. Smallest pollen grains of about 5 x 2.4 µm is noted in Myosotis palustris and some members of Boraginaceae, while the largest pollen grains (> 200 µm in diameter) are observed in Curcurbitaceae, Nyctaginaceae and Orectanthe ptaritepuiane (Abolbodaceae).

In taking measurements of size the length of polar axis (PA), equatorial diameter (ED) and sometimes equatorial breadth (EB) are considered in bilateral grains.

In radially symmetrical pollen grains the PA and the greatest ED can be measured in equatorial view, while the EB can be measured in polar view only. It is also necessary to measure exine elements, taking into consideration the thickness of exine, sexine/nexine thickness ratio and the thickness of the exine projections greater than 0.5 µm if any.

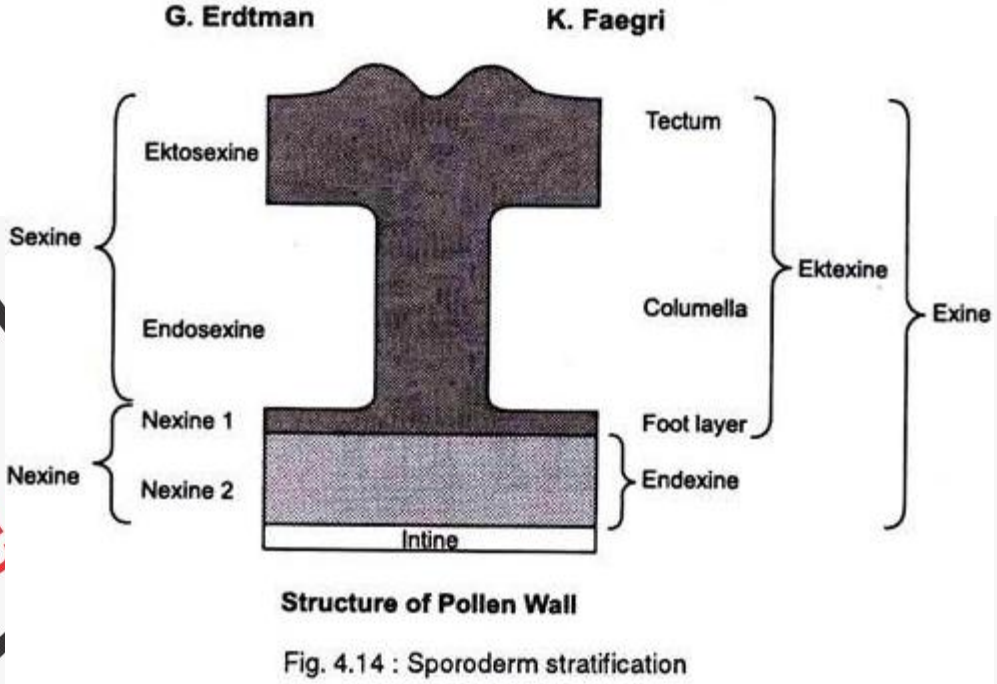
Erdtman (1945) categorized the different pollen size classes based on the size expressed as length of the longest axis (Table 4.2).

Table 4.2 : Pollen size classes (*after* Erdtman, 1945).

	Pollen size class	Length of longest axis
1.	Very small grains (*Sporae perminutae*)	<10µm
2.	Small grains (*Minutae*)	10 - 25 µm
3.	Medium sized grains (*Mediae*)	25 – 50 µm
4.	Large grains (*Magnae*)	50 – 100 µm
5.	Very large grains (*Permagnae*)	100 – 200 µm
6.	Gigantic grains (*Giganteae*)	> 200 µm

CHARACTERISTIC # 8. POLLEN WALL OR SPORODERM STRATIFICATION:

The pollen wall, the sporoderm is generally stratified i.e. layered (Fig. 4.14). The walls of the mature pollen, at least in angiosperms, consists of two fundamentally different layers, intine and an outer acetolysis resistant layer exine composed of sporopollenin.



Structure of Pollen Wall

Fig. 4.14 : Sporoderm stratification

The exine covers the entire pollen surface except germinal apertures where it is absent or greatly reduced. The exine of pollen grains can be divided into an outer sculptured sexine and an inner unsculptured nexine (Fig. 4.14).

Sexine again consists of two layers: the outer, ectosexine and inner, endosexine. The sexine is generally constituted of a set of radially-directed rods supporting a roof-like structure (tectum or tegillum), which may be partially perforated or completely absent.

Rods supporting the tectum are known as columella, and rods not supporting anything but standing vertically on the nexine are called bacula. Columella are usually simple, but may be branched. In Compositae the columellae are either distally branched (digitate) or proximally branched (conjunctate) or sometimes the columella hang down from the tectum, e.g., Caryophyllaceae (Fig.4.15).

The nexine has been divided into two layers namely nexine I and nexine II. Knut Faegri (1964) proposed an alternative terminology for exine stratification (Fig. 4.14).

He recognized two layers of exine, the outer ektexine (including sexine and nexine I) and endexine (nexine II). He designated nexine I as foot layer and considered it to be the basal part of ektexine for its identical chemical composition and staining property as that of sexine.

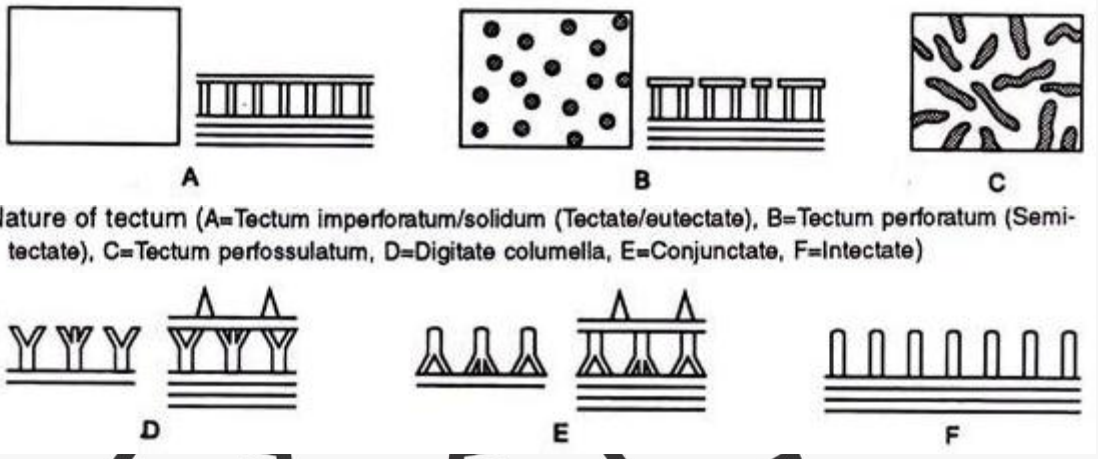


Fig. 4.15 : Nature of tectum (A=Tectum imperforatum/solidum (Tectate/eutectate), B=Tectum perforatum (Semi-tectate), C=Tectum perfossulatum, D=Digitate columella, E=Conjunctate, F=Intectate)

Faegri's ektexine is quite different from the endexine because the former contains more dense sporopollenin and stains more deeply. The ektexine may be regarded as a three-layered structure in which the granules form small columns, columella, thus dividing an outer tectum and an inner foot layer strata. The endexine is often well developed in dicots, but is virtually absent or have it only in the apertural region in monocots.

In some pteridophyte spores (e.g. Polypodiaceae) and a few gymnosperms pollen (e.g., *Taxodium*) there is a hyaline loosely organized sporopolleninous envelope called Perine covering the exine (Fig. 4.16). The perine maybe continuous or sometimes folded in various ways. In some gymnosperms, especially among conifers, the ektexine enlarges to form bladdery wings (*Saccus*, P1.Sacci) generally two (*Pinus, Cedrus, Abies, Picea* etc.) or one (*Tsuga*) in number.

EXINE ORNAMENTATION:

There are two different types of exine ornamentation, the structure or texture and the sculpturing. The structure comprises of all the internal (infratectal) baculae of various form and arrangements. All the ektexine characters belong to the structural features, while the sculpturing comprises external (supratectal) geometric features without reference to their internal construction.

Intine and Exine:

The protoplasm of the pollen grains is enclosed by a wall made of intine and exine. The intine is a hyaline layer. The exine (Fig. 6) consists of an inner homogeneous layer (called endine or nexine and an outer heterogeneous layer (called ectine or sexine).

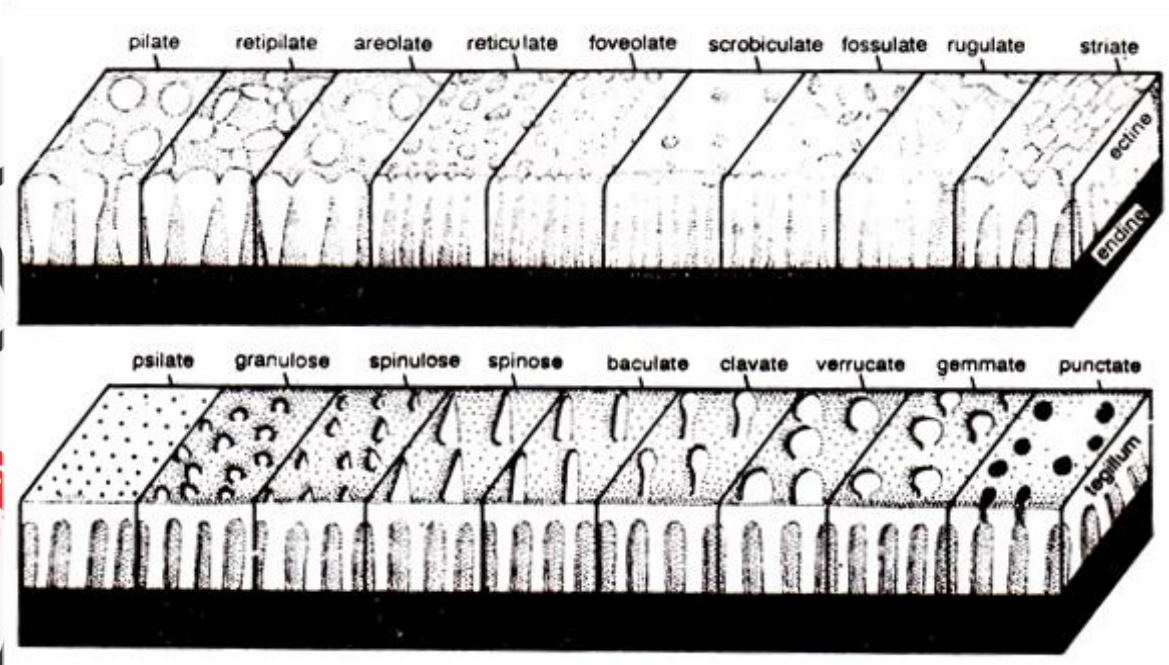


Fig. 6. *Exine strata and surface patterns of pollen grains.*

Ectine and Endine:

Ectine is the outer layer of exine while endine is the inner layer of exine.

Columellae and Tegillum:

The radial rods which form the ectine are called columellae. The columellae are either free at their tips or are fused to form a layer called tegillum

Exine Ornamentation:

Various types of ornamentation patterns are shown by exine surface. As mentioned above, the ectine of exine is composed of radial rods or columellae. When the distal surfaces of columellae are bright and the intervening regions are dark, the pattern is called pilate (Fig. 6). The columellae in most of the grains are fused to form different types of patterns having depressed areas (called lumina) and the intervening areas between lumina (called muri).

When a network is produced by lumina and muri it is called reticulate. In the reticulate pattern, if there is the incomplete fusion of columellae, it is called retipilate, if there are circular and closely placed lumina it is called foveolate, if there are circular but distantly placed lumina it is called scrobiculate, if lumina are elongated it is called fossulate, if lumina are parallel it is called striate and; if lumina are anastomosing it is called rugulate.

The exine ornamentation is called areolate when luminoid network surrounds islands of raised areas. If the excrescences (outgrowths) on the exine are in the form of very minute granules the pattern is called granulose, if the excrescences are in the form of spinules with pointed or blunt ends it is called spinulose, if the excrescences are in the form of rounded warts with constricted base it is called gemmate, and if the bases are not constricted it is called verrucate.

When the outgrowths on the exine are in the form of tubercles it is called tuberculate, if these are long with pointed ends the pattern is called spinose, if outgrowths are rod shaped it is called baculate, and when they are club shaped it is called clavate.

NPC SYSTEM OF CLASSIFICATION OF POLLEN GRAINS:

G. Erdtman (1969) proposed NPC-System pollen/spore classification based on the apertures, their Number (N- whether single or two or many), Position (P- polar: distal or proximal; global; meridional) and Characters (C – circular or elongated) with regard to microspore tetrad (Fig. 4.7). Under this system the term 'treme' (aperture) has been used for preparing keys for the classification of the pollen grains/spores.

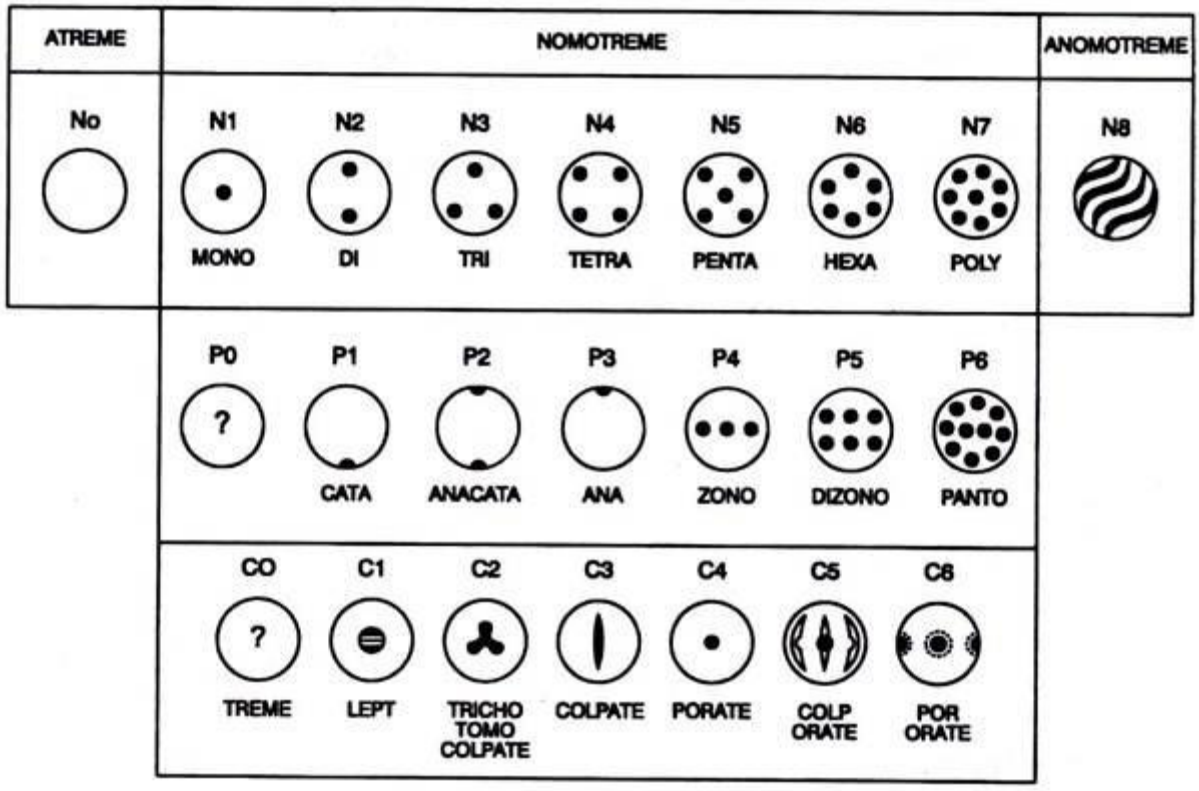


Fig. 4.7 : NPC classification of pollen (*after* Erdtman, 1969)

The pollen number (N) groups are of nine types. The grain without aperture is named "Atreme" and is designated as No. Depending upon the number of apertures, the types of pollen are Monotreme (N_1) with one aperture, Ditreme (N_2) with two apertures; Tritreme (N_3) with three apertures, Tetratreme (N_4) with four apertures, Pentatreme (N_5) with five apertures, Hexatreme (N_6) with six apertures and Polytreme (N_7) having more than six apertures. Irregularly arranged spiral apertures over the surface of the pollen irrespective of their number are designated as 'Anomotreme' (N_8).

On the basis of the position (P) of apertures, pollen are categorized into seven groups (P_0 to P_6). In 'Catatreme' (P_1) pollen aperture is in proximal face, while in 'Anatreme' (P_3) it is in distal face. The pollen are designated as Anacatatreme' (P_2) where apertures are both in proximal and distal faces.

The pollen grains are referred to as 'Zonotreme' (P_4), when the apertures are located on the equatorial zone. 'Dizonotreme' (P_5) are like zonotreme, but with two rows of apertures on the equatorial region. In 'Pantotreme' (P_6), apertures are globally distributed all over the pollen surface.

Like position groups the character (C) groups are of seven types (C_0 to C_6). If the character of the aperture is not known, it is designated as C_0. Pollen having an aperture like thin area or Leptoma is designated as C_1. Pollen with one leptoma is called Monolept, it may be called Catalept if present in the proximal face, or Analept if in the distal face.

Pollen with three- slit like colpus are called Trichotomocolpate which belongs to C_2 category. The remaining character classes i.e., C_3, C_4, C_5 and C_6 include Colpate (with colpa i.e. furrow), Porate (with pore i.e. circular aperture), Colporate (both with colpa and pore/ora apertures), Pororate (aperture with pore and ora) respectively.

Based on NPC classification, each pollen type is designated by using a three digit number (Fig. 4.8). The first digit denotes the number of apertures, for example, 100 is assigned to monotreme, 200 to ditreme, 300 for tritreme, 400 for tetratreme, 500 for pentatreme, 600 for hexatreme, 700 for polytreme, and 8 for anomotreme and 9 for atreme.

The second digit denotes the position of the aperture, e.g. 010 to proximal aperture, 030 for distal aperture, 040 for equatorial aperture, 060 for global aperture. The third digit denotes the characters of the aperture, e.g., 002 for trilete, 003 for colpate, 004 for porate, 005 for colporate. Therefore, the number 112 is assigned to trilete grains, similarly 133 to monosulcate grains, 343 to tricolpate and 345 to tricolporate grains, etc. (Fig. 4.8).

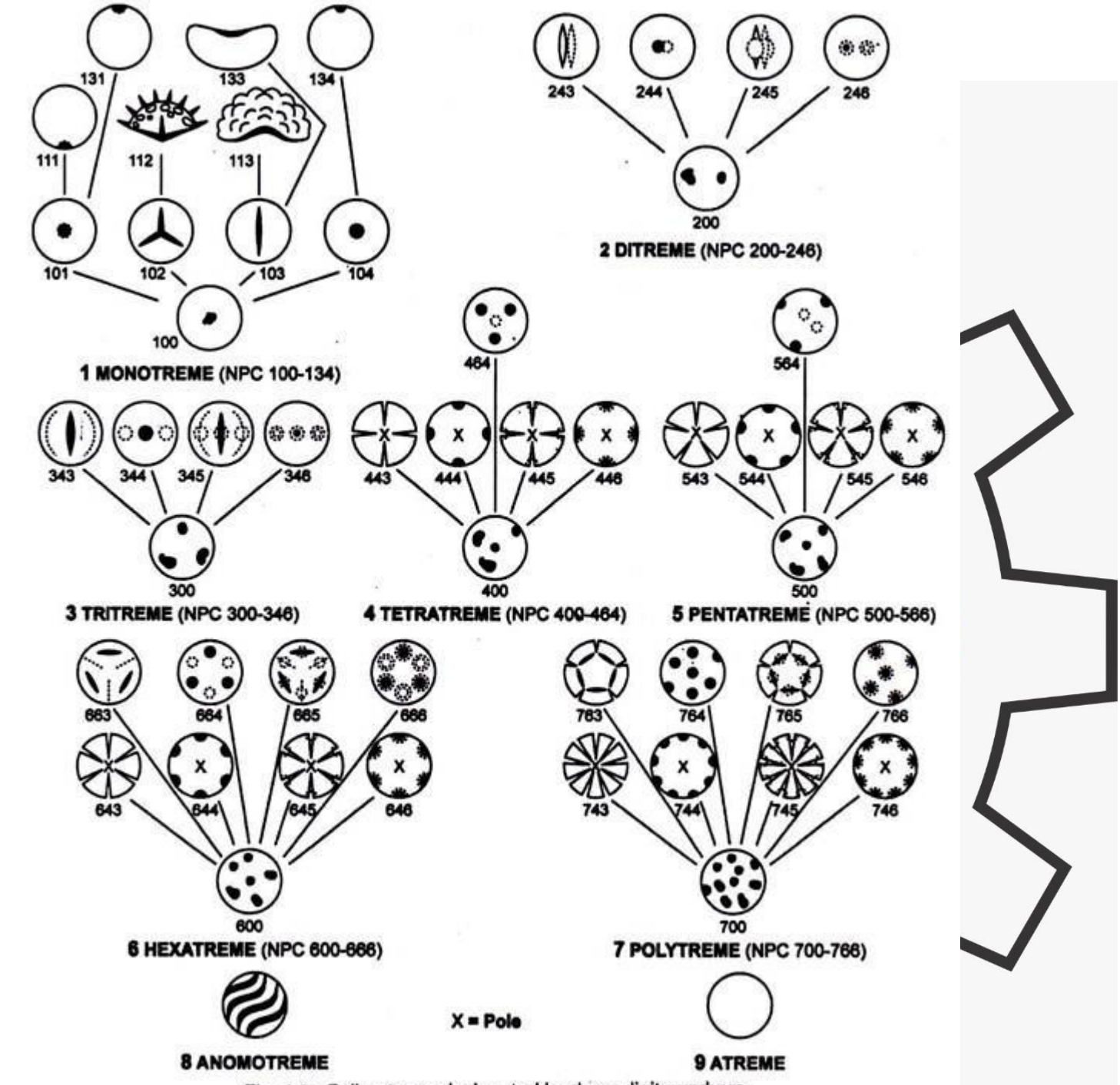


Fig. 4.8 : Pollen types designated by three digit numbers

APPLIED PALYNOLOGY

Top Nine Branches Of Palynology

The branches are: 1. Palynotaxonomy 2. Aeropalynology 3. Melissopalynology (= melittopalynology) 4. Forensic Palynology 5. Palaeopalynology 6. Copropalynology (Greek 'kopros' means dung) 7. Entomopalynology 8. Palynodebris 9. Latropalynology.

Branch 2. Aeropalynology:

Aeropalynology is the study of palynomorphs found in the atmosphere. The term palynomorph encompasses pollen, spores and other bioparticles that are acid resistant.

The study also includes their eventual dissemination, deposition and impact on human systems.

Pollen grains are dispersed more than 400 miles away from the source plants. They can be found more than two miles above the surface. The airborne pollen grains originate from anemophilous plants and so they are small, light, smooth walled, colourless, produced in large numbers, dry and lack nectar

The first and foremost requirement in the study of airspora is to identify and detect the airborne palynomorphs and sporomorphs. A sound knowledge of ground flora is essential for this purpose. The study of ground flora of a particular area will reveal the genera and species present in that locality and their flowering season.

Pollen grains from each species are to be studied and the slides of them are to be deposited in herbarium for future references.

The airborne pollen are trapped for sampling by two major principles:

(1) Simple gravimetric method where pollen and spores are deposited by normal gravitational force on slides, and

(2) Suction method where the atmospheric air is sucked in with the help of instruments called sampler.

There are many types of sampler that can suck certain volume of air according to a known velocity for a definite time. Along with the air pollen and spore are sucked in and they get stuck either on a plastic band or sticky cello-tape (fitted according to sampler), which were previously placed there for the purpose of trapping pollen.

Mention may be made of Burkard seven days sampler, Burkard personal slide sampler, Burkard petriplate sampler, Anderson sampler and Hirst spore trap.

The next task is to analyze the trapped grains. Analysis includes identification and quantification of sporomorph and palynomorphs. Microscopic identification is dependable in most cases. Certain fungal spores cannot be identified under the microscope. If the spores are culturable, they are isolated and cultured in cultural media. Colonies are formed and thus they are identified.

Pollen and spores are identified on the basis of sporoderm stratification, shape, size and apertural characters. The morphological characters of trapped pollen and spore are compared with those of ground flora and thus the airborne grains get identified. The frequency of the identified grains is also calculated.

Trapping, identification and quantification are carried out throughout the year and the results are recorded. The data help to prepare the pollen calendar of a particular locality. A pollen calendar reveals the name of plant species that release pollen and spore in particular month(s). It also reveals the approximate amount of grains released in that season.

The term 'pollen count' is frequently used to represent the concentration of pollen in atmosphere. It is a measure of how much amount of pollen and spore is present in the atmosphere in a particular area at specific time. The count may consist of a particular type of pollen or spore, or all the pollen grains and spores present in the air. Pollen count is expressed as the number of pollen present in a cubic metre or other standard volume of air over a twenty-four hour period at a particular place. The samplers collect pollen grains.

A sampler has a drum or rod coated with silicone grease or sticky cello-tape. During trapping the rod/drum is rotated one turn only in an entire 24 hours of a day with the help of a motor fitted with the sampler. The trapped pollen grains are then analyzed for identification and quantification.

Anemophilous plants of local vegetation determine the frequency of airspora and so the pollen counts.

Meteorological factors also influence the pollen count. The change in climatic condition has a profound effect on the concentration of air spora.

Pollen count increases in dry, warm and breezy days. It decreases in chilly with high humidity and rainy days. Moreover pollen count can be changed due to pollution, industrialization, population growth, tree plantings and cuttings, land use etc.

Singh et al. (2004) published a detailed list of airspora of the various parts of India along with their sources in literature. Chanda and Sarkar (1972) reported the existence of the following pollen at the atmosphere of greater Kolkata. The grass pollen contributed 39% to the total pollen present in air.
The dominant pollen types are from *Amaranthus, Argemone, Azadirachta indica, Caesalpinia, Carica papaya, Chenopodium, Mangifera indica, Xanthium strumarium* etc. In West Bengal 59 types of pollen in air were reported. In the month of May the concentration of pollen was maximum. The maximum concentration of pollen grains belonging to the family Asteraceae and Chenopodiaceae was found in the month of June.
Pollen grains have impact on human systems. They cause allergy on sensitive individuals. The term allergy is defined as **"an altered or accelerated reaction of a person to a second or subsequent exposures to a substance, usually harmless to the general population, to which he/she has been sensitized during the first exposure".** Hay fever or allergic rhinitis is the best known allergy of all. Hay fever is caused by pollen grains of *Betula, Populus, Salix* and *Chrysanthemum* etc. Allergy is also caused by the pollen grains of *Cynodon dactylon, Amaranthus spinosa, Chenopodium album, Cannabis* sativa and *Cassia occidentalis* etc.
The symptoms of hay fever are sneezing, clogged nose, itching nose, throat and eye, conjunctivitis and watering eyes. Aeropalynology comes under the purview of aerobiology that includes the study of bioparticles present in the atmosphere in addition to pollen and spore. In aerobiological research palynologists in collaboration with clinicians search the causative agents present in the atmosphere that have impact on human health.

Branch 3. Melissopalynology (= melittopalynology):

Melissopalynology is the study of pollen and spore present in honey. By extension, it also includes honeydew elements (HDE), i.e. fungal spores, hyphae and microscopical algae and other bioparticles. 'Melissa' and 'melitta' mean a bee. *Apis mellifera* is the scientific name of common honeybee. In Latin the word *mellifera* means honey. In addition to *Apis mellifera* L. the other Indian honeybees are *A. indica* F., *A.florea* F. and *A. dorsata* F.

Honeybee requires nectar, pollen, resin and water for their survival. The bees are adopted for feeding on nectar and pollen. Resin reinforces the hive and water cools the hive. Moreover water dilutes the honey that is fed to larvae. In a hive there are three types of bee: the single queen bee, a variable number of male bee or drone and 20000 to 40000 or more worker bees.
The worker bees travel long distances to collect nectar. They suck nectar from nectary present in the flower. The nectar is stored in the crop of bee. Regurgitation of nectar starts before the return of bees. On return to the hive the other worker bees remove the nectar. The foraging bees and other worker bees regurgitate the nectar.
Regurgitation occurs a number of times till the nectar is transformed into honey. During the process the natural complex sugar (sucrose) of the nectar is converted to simple sugar (fructose, glucose etc.) by the enzymes. The sugary fluid is then stored in honeycomb, which is left unsealed. The honeycomb is a group of hexagonal cells composed of beeswax and propolis.
The sugary fluid is high in water content. The formation of ripe honey is accomplished by evaporating the excess water. This happens when the bees inside the hive fan their wings thus circulating air across the honeycomb. The reduction in water content causes the increase of sugar concentration. The honeycomb containing the ripe honey is then capped (sealed) with beeswax and propolis.

Honey is thick, sweet syrupy, white or yellowish or brown or other colours, and viscid fluid. Honeybees from the nectar of flowers produce it. Honey is consumed primarily as an energy source and is stored in the honeycomb for unfavourable situation like winter etc. Pollen grains are used as the primary source of protein and other nutrients.
Honey is composed of 14-18% of water, 76-80% of glucose, fructose, levulose, dextrose etc., pollen, mineral salts (compounds of calcium, potassium, sodium, magnesium, copper, silica, phosphorus, manganese, silicon, iron etc.), acids (malic, tartaric, citric, succinic etc.), plant pigments like carotene, xanthophyll and chlorophyll; enzymes like invertase, diastase, catalase etc., vitamins (B_1, B_2, K, folic acid, biotin, pyridoxine, pantothenic acid etc.) and amino acids (cystine, lysine, glycine, aspartic acid, glutamic acid, alanine, valine etc.).
The composition varies and it is dependent upon the flower that provided the nectar. The variation in colour is also dependent on the plant that foraged the bee, i.e. alfalfa and clover produce white honey and Acacia produces straw colour honey. The honeys produced from cotton, Plectranthus and Eucalyptus are also white.
Golden or light yellow coloured honeys are obtained from litchi, mustard and rubber. Honey is significantly sweeter than table sugar. It was almost the sole sweetening agent from ancient times until cane sugar became

commercially important. Honey being complete natural and biological product and free from any type of impurities, it is often preferred over sugar.

It is one of the best health food consumed by man. Honey has become a common ingredient in many types of ayurvedic medicines as it is found to contain certain types of medical benefits. Apart from food, honey has always been held in high regard. In language, literature and culture honey is frequently used as a symbol that is supposed to bring good luck or to keep away evil. It is the symbol of sweetness of every kind.

The honey does not spoil easily. Due to the presence of low water and high sugar concentration, it does not allow to grow any airborne yeast or bacteria. Yeast/bacteria get plasmolyzed when they come in contact with concentrated sugary honey. So long the moisture content remains below 18% almost no microorganisms can multiply in honey.

Pollen grains are collected during the visit of plants by bees for nectar. The legs of bee help in this process.

Pollen analysis of honey samples reveals the followings:

a. It provides the plant species foraged by honeybees.

b. The identified plants provide the fingerprint of environment and pollen spectrum from where the honey comes.

c. It provides the botanical and geographical origin of honey.

d. It is an indispensable tool to authenticate honey-unifloral honey or multifloral honey. If unifloral whether the honey sample contains pollen grains of the same plant as is declared. As for example – *Brassica*-honey, Acacia-honey and Citrus-honey etc.

e. It detects whether honey sample contains allergenic pollen grains.

f. It detects whether honey sample contains poisonous pollen, e.g. *Datura* and *Rhododendron* etc.

g. It identifies pollen that are toxic to bees and cause bee poisoning, e.g. Kalmia, *Cuscuta*, *Tilia* and *Solanum* etc.

EXPERIMENTAL EMBRYOLOGY-

Modern embryology seems to comprise three main disciplines. The first, or descriptive embryology, is a study of the various developmental processes that take place in a plant from the initiation of the sex organs to the maturation of the embryo. The second, or phylogenetic embryology, attempts to evaluate these data in determining the interrelationships of the different orders and families with a view to improving the existing schemes of classification. The third, or experimental embryology, is concerned with an imitation and a modification of the course of nature, with a view to understanding the physics and chemistry of the various processes underlying the development and differentiation of the embryo, so as to bring them under human control to the furthest extent possible.

It is now generally considered that every living cell in a plant possesses the potentially of producing an entire new plant or in other words it is totipotent. This phenomenon of capability of such a totipotent cell in generating an entire new plant is called as totipotency.

Explant:
An excised piece of differentiated tissue or organ is regarded as an explant. The explant may be taken from any part of the plant body e.g., leaf, stem, root.

Callus:
The unorganized and undifferentiated mass of plant cells is referred to as callus. Generally, when plant cells are cultured in a suitable medium, they divide to form callus i.e., a mass of parenchymatous cells.

Dedifferentiation:
The phenomenon of mature cells reverting to meristematic state to produce callus is dedifferentiation. Dedifferentiation is possible since the non- dividing quiescent cells of the explant, when grown in a suitable culture medium revert to meristematic state.

Re-differentiation:
The ability of the callus cells to differentiate into a plant organ or a whole plant is regarded as re-differentiation.

Totipotency: The ability of an individual cell to develop into a whole plant is referred to as cellular totipotency. The inherent characteristic features of plant cells namely dedifferentiation and re-differentiation are responsible for the phenomenon of totipotency.

BASIC TECHNIQUE OF PLANT TISSUE CULTURE:

The general procedure adopted for isolation and culture of plant tissues is depicted in Fig. 42.4

The requisite explants (buds, stem, seeds) are trimmed and then subjected to sterilization in a detergent solution. After washing in sterile distilled water, the explants are placed in a suitable culture medium (liquid or semisolid form) and incubated. This results in the establishment of culture. The mother cultures can be subdivided, as frequently as needed, to give daughter cultures.

The most important aspect of in vitro culture technique is to carry out all the operations under aseptic conditions. Bacteria and fungi are the most common contaminants in plant tissue culture. They grow much faster in culture and often kill the plant tissue.

Further, the contaminants also produce certain compounds which are toxic to the plant tissue. Therefore, it is absolutely essential that aseptic conditions are maintained throughout the tissue culture operations. Some of the culture techniques are described here while a few others are discussed at appropriate places.

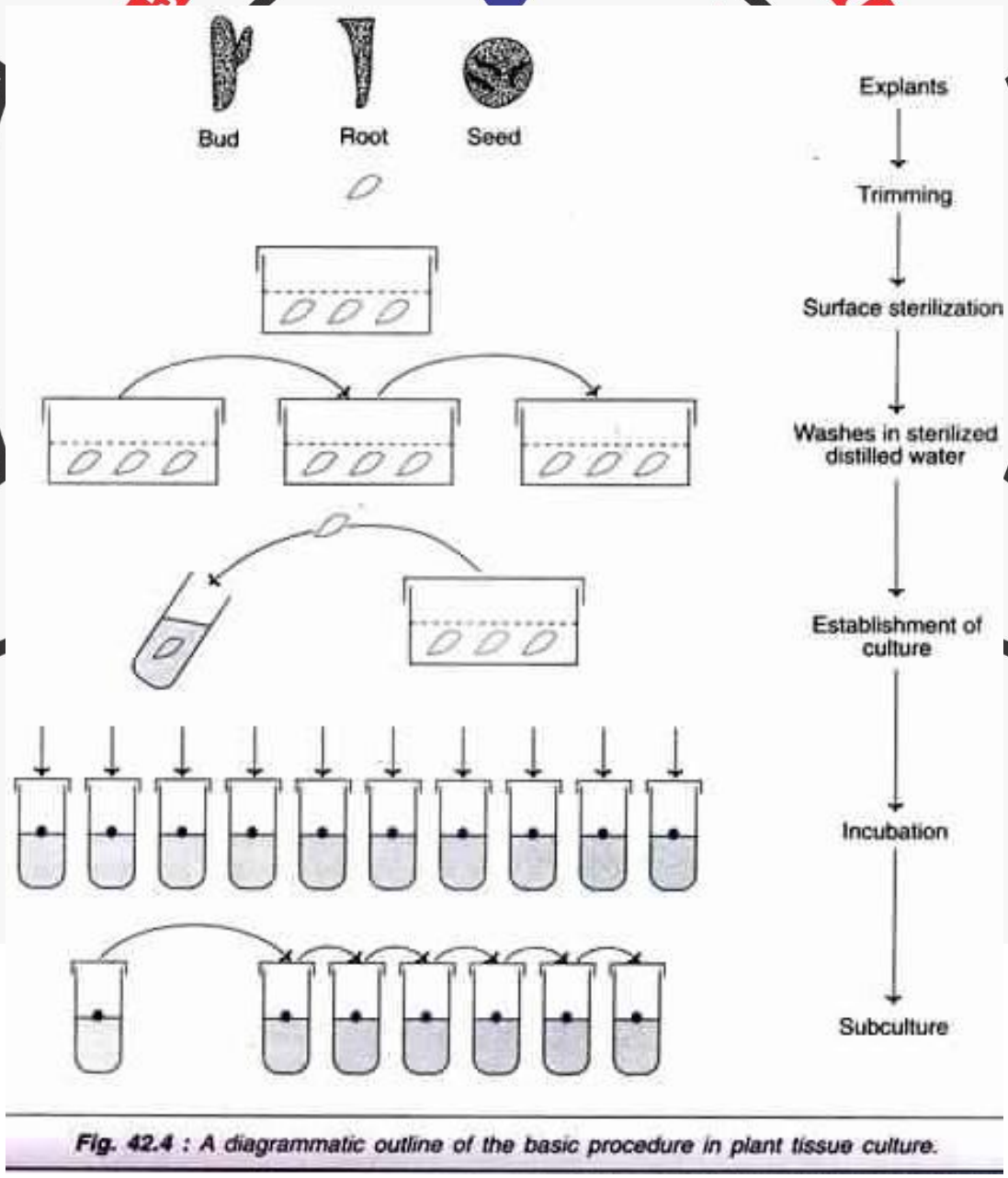


Fig. 42.4 : A diagrammatic outline of the basic procedure in plant tissue culture.

CULTURE MEDIA:

The formulation or the medium on which the explant is cultured is called culture medium. It is composed of various nutrients required for proper culturing. Different types of plants and organs need different compositions of culture media. A number of media have been devised for specific tissues and organs.

Some important of them are:

MS (Murashige and Skoog) Medium, LS (Linsmaier and Skoog) Medium, B5 (Gamborg's) Medium, White's Medium, etc.

Important constituents of a culture medium are:

Organic supplements:

(a) Vitamins like thiamine (B_1), Pyridoxin (B_6), Nicotinic Acid (B_3), etc.
(b) Antibiotics like Streptomycin, Kanamycin;
(c) Amino Acids like Arginine, Asparagine.

(ii) Inorganic Nutrients:

Micronutrients as Iron (Fe), Manganese (Mn), Zinc (Zn), Molybdenum (Mo), Copper (Cu), Boron (B).
Macronutrients include six major elements as Nitrogen (N), Sulphur (S), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg).

(iii) Carbon and Energy Source:

Most preferred carbon source is Sucrose. Others include lactose, maltose, galactose, raffinose, cellobiose, etc.

(iv) Growth Hormones:

a. Auxins-mainly for inducing cell division.
b. Cytokinin-mainly for modifying apical dominance and shoot differentiation.
c. Abscisic Acid (ABA)-Used occasionally.
d. Gibberellins-Used occasionally.

Gelling Agents:

These are added to media to make them semisolid or solid. Agar, Gelatin, Alginate etc. are common solidifying or gelling agents.

Other Organic Extracts:

Sometimes culture media are supplemented with some organic extracts also like coconut milk, orange juice, tomato juice, potato extract, etc.

Composition of MS & White's media

Table : Stock solutions of MS basal medium

Constituents	Quantity (gm/litre)
Stock Solution I	
$MgSO_4.7H_2O$	7.400
KH_2PO_4	3.400
KNO_3	38.000
NH_4NO_3	33.000
$CaCl_2.2H_2O$	8.800
Stock Solution II	
H_3BO_3	1.240
$MnSO_4.4H_2O$	4.460
$ZnSO_4.7H_2O$	1.720
$Na_2MoO_4.2H_2O$	0.050
$CuSO_4.5H_2O$	0.005
$CoCl_2.6H_2O$	0.005
Stock Solution III	
$FeSO_4.7H_2O$	5.560
$Na_2.EDTA.2H_2O$	7.460
Stock Solution IV	
Inositol	20.000
Thiamine HCl	0.100
Pyridoxine HCl	0.100
Nicotinic acid	0.100
Glycine	0.400

1. White's Culture Medium

Component	*Quantity (mg/litre)*
(a) *Inorganic Components*	
KNO_3	80
$Ca(NO_3)_2.4H_2O$	260
KCl	65
$MgSO_4.7H_2O$	360
Na_2SO_4	200
$NaH_2PO_4.2H_2O$	165
$MnSO_4.4H_2O$	3
H_3BO_3	0.5
$ZnSO_4.4H_2O$	0.5
$Na_2MoO_4.2H_2O$	0.05
$CoCl_2.6H_2O$	0.025
Fe $(C_6H_5O_7).3H_2O$	10
(b) *Organic Components*	
Niacin	1.25
Glycine	7.5
Pyridoxine HCl	0.25
Thiamine	0.25
Calcium Pentothenate	0.25
Sucrose	20.00

ANTHER CULTURE OR ANDROGENESIS:

The technique of production of haploids through anther or microspore culture is termed as androgenesis. It is a method par excellence for the large scale production of haploids through tissue culture.

Androgenesis technique for haploid production is based on the in-vitro culture of male gametophyte i.e., microspore of a plant resulting into the production of complete plant from it. It is achieved either by anther culture or by microspore (pollen) culture.

The technique of another culture is quicker for practical purposes and is an efficient method for haploid production.

But sometimes during another culture, the plantlets may originate from different other parts of anther also (along with from the pollens). On the other hand, microspore culture is free from any uncontrolled effects of the anther wall or other tissues. Microspore culture is ideal method for studying the mutagenic and transformation patterns (Fig. 9).

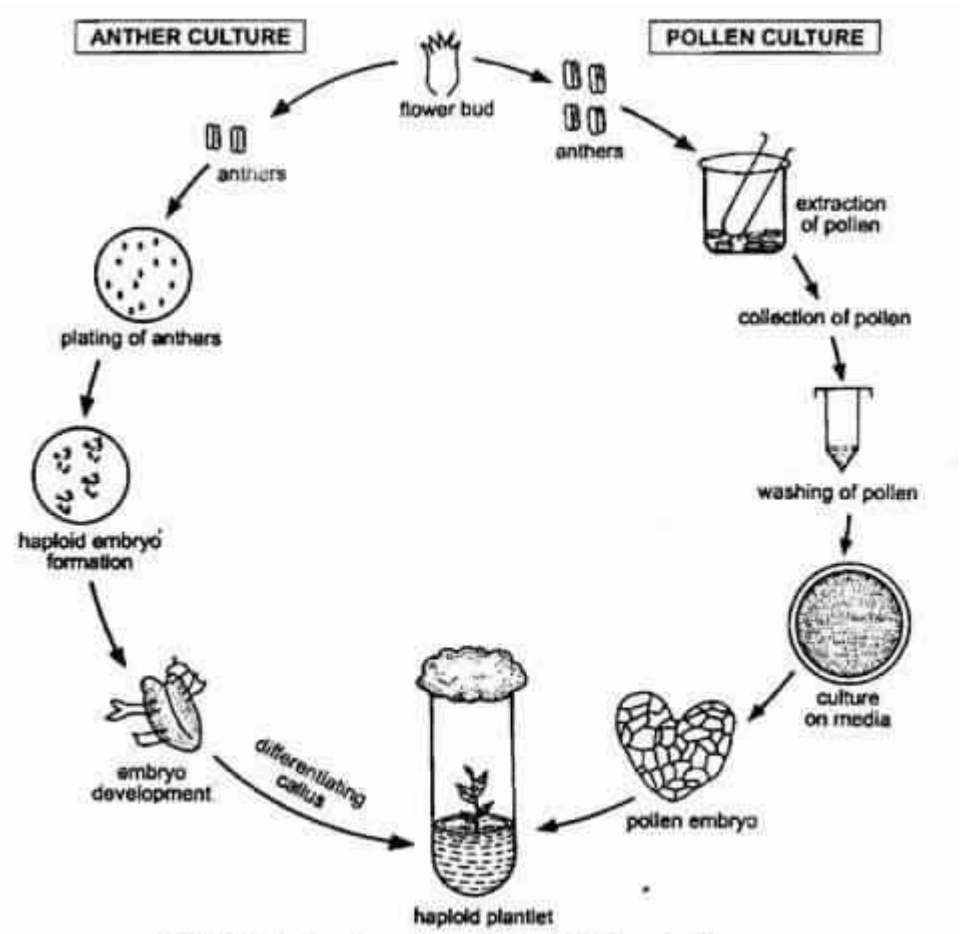


Fig. 9. Androgenesis for Haploid Production.

EMBRYO CULTURE:

The technique of embryo culture involves the isolation and growth of an embryo under in-vitro conditions to obtain a complete viable plant. First success for embryo culture was made by Hannig in 1904 when he isolated and cultured embryos of two crucifers namely *Cochleria* and *Raphanus*. Embryo culture is used widely in the fields of agriculture, horticulture and forestry for production of hybrid plants.

Embryos can be used as explants to generate callus cultures or somatic embryos. Both immature and mature embryos can be used as explants. Immature, embryo-derived embryo genic callus is the most popular method of monocot plant regeneration.

This technique allows the detailed study about the nutritional requirements of embryos during different developmental stages. Also, it helps for identifying the regeneration potential of embryos. Embryo culture is advantageous for in-vitro micro propagation of plants, overcoming seed dormancy and for production of beneficial haploid plants.

Source Material:

Physiologically uniform embryos of the same size and same growth phase should be used for culture, which can be achieved if the plants are raised and maintained under controlled condition.

For supply of materials regularly the selected plants should bear the flowers at regular intervals; to obtain the embryos of a specific age, artificial pollination of freshly opened flowers is necessary and it may be desirable to prepare a chart showing the different stages of embryo development with days after pollination.

Where the embryos become abortive after development of seeds, the embryos should be excised before the onset of abortion.

Surface Sterilization:

As the embryo grows within the seed which is within a totally sterile environment, so surface sterilization of the embryo is not needed unless there is an injury on seed-coat, or any kind of systemic infection is present.

Otherwise, mature seeds, entire ovules or fruits are surface sterilized and the embryos are removed aseptically from the surrounding tissue. In case of orchid seeds the entire seed contains the morphologically undifferentiated spherical embryo. As there is no nutritive tissue and the seed coat is very thin, the whole seed is cultured in place of embryo.

Excision of Embryo:

The embryo-excision operation is performed aseptically in a laminar air flow cabinet with the help of a stereomicroscope, and using the dissecting tools like forceps, needles, scalpels, razor blades, etc.

If the seed coat is hard then seeds are soaked in water and cotyledons are splitted open and embryos are excised. But in case of immature embryos where these are embedded in endosperm, the incision is made at the micropylar end and pressure is applied to isolate the young embryo.

Nutritional Requirements in Culture Medium:

There are mainly two phases of embryo development, heterotrophic phase—where the embryo is dependent on the nutritional tissue or maternal tissue, another is the autotrophic phase—where the embryo is metabolically capable of synthesizing substances required for growth, i.e., becoming independent for nutrition.

The media composition for embryo culture differs for young or immature embryos from those of mature embryos. When immature or heterotrophic embryos are cultured, the medium requirement becomes progressively complex to permit the expression of the total developmental potential of an embryo.

This is dependent upon the progressive activation of critical enzyme systems or biochemical pathways concerned with the synthesis of substances necessary for the growth of embryos at a specific age. But the embryos of mature stage are completely autotrophic and grow on a simple medium comprising of mineral salts with carbohydrate energy source.

Mineral salts are used according to MS, B5 or White's media with some modifications. Among the carbohydrates, sucrose is the main source, addition of maltose, lactose, raffinose or mannitol may be required for some species of embryo culture. The carbohydrate concentration may also vary according to the maturity of embryo.

Presence of ammonium salt has been found essential for proper growth and differentiation of embryos. Various amino acids or their amides are sometimes essential for embryo growth. Casein hydrolysate (CH), an amino acid complex has been widely used as an additive to embryo culture media.

Coconut milk helps the young excised embryos to increase in length of various species like sugarcane, barley, tomato, carrot, or some interspecific hybrids. Among the growth regulators, GA is used at low levels which effectively stimulate excised heart shaped embryos.

The incubation temperature for embryonic growth and germination may vary with the genotype and species. Initial dark incubation for 4 days of embryo culture is essential, following which these can be grown to maturity under light condition according to requirement.

The protocol of embryo culture has been depicted in Fig. 19.1.

Morphogenesis in Partially Differentiated Embryo:

Embryo development is a process from zygote formation to embryo germination, the stages can be classified into five stages:

Stages:

I. Cleavage and differentiation:
Cell division with little growth; differentiation of all major tissues.

II. Growth:
Rapid cell expansion and division.

III. Maturation:
Little or no cell division or expansion, synthesis and storage of reserve materials.

IV. Dormancy:
Developmental arrest.

V. Germination:
Renewed cell expansion and division; embryo growth.

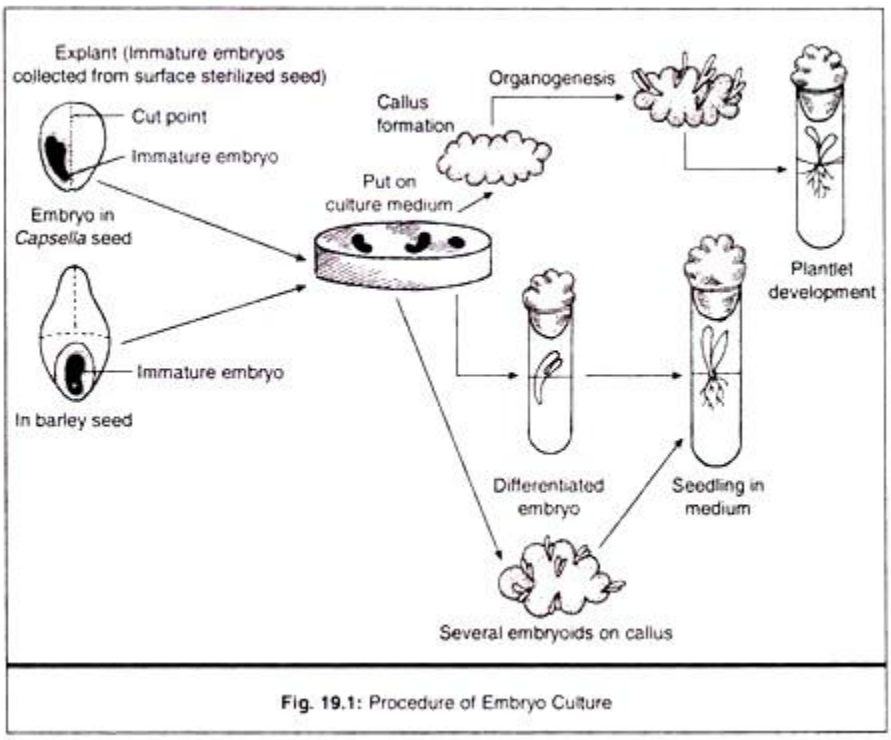


Fig. 19.1: Procedure of Embryo Culture

Detailed cultural studies have revealed the role of suspensor in embryo development. Suspensor is an ephemeral structure which is found at the radicular end of the pro-embryo that attains its maximum development by the time the embryo reaches the globular stage. In some cases the presence of suspensor with the immature embryo is essential for maturity. The requirement of the suspensor may be substituted by the addition of GA or ABA to the culture medium.

After full development of the embryo it enters into a phase of dormancy characterised by metabolic quiescence and development arrest of cells. Dormancy may last for different time periods for different kinds of crop species. Excised immature plant embryos on a nutrient medium tend to bypass the stage of dormancy and cease to undergo the linear embryogenic mode of development. The phenomenon of seedling formation without completing normal embryogenic development is called precocious germination. Casein hydrolysate, high sucrose level, extension, elevated temperature; high light intensity and also ABA like substances prevent precocious germination of embryo. In many plant species fully developed seeds contain embryos that lack differentiation into radicle, plumule and cotyledons. In these cases the globular stages are attained and the embryonal end proximal to the micropyle regarded as the 'radicular pole' and distal to the micropyle is regarded as the 'plumular pole'. In case of orchid, the plumular pole enlarges to form a spherical structure which turns green and after attainment of certain size differentiates into roots and shoots.

PROTOPLASTS CULTURE:

What is a Protoplast?

It is known that each and every plant cell possesses a definite cellulosic cell wall and the protoplast lies within the cell wall except some reproductive cells and the free floating cells in some fruit juices like coconut water.

Therefore, protoplast of plant cell consists of plasma-lemma and everything contained within it.

But those of importance to plant protoplast culture are produced experimentally by the removal of cell wall by either enzymatically or mechanical means from the artificially plasmolysed plant cells. Experimentally produced protoplasts are known as isolated protoplasts.

Therefore, isolated protoplast is only a naked plant cell surrounded by plasma membrane—which is potentially capable of cell wall regeneration, cell division, growth and plant regeneration in culture.

Different Sources of Plant Tissue and Their Condition for Protoplast Isolation:

Protoplast can be isolated either directly from the different parts of whole plant or indirectly from in vitro cultured tissue. Convenient and suitable materials are leaf, mesophyll and cells from liquid suspension cultures. Protoplast yield and viability are profoundly influenced by the growing conditions of plants serving leaf mesophyll sources. The age of the plant and of the leaf and the prevailing conditions of light, photoperiod, humidity, temperature, nutrition and watering are contributing factors. Cell suspension cultures may provide a more reliable source for obtaining consistent quality protoplasts. It is necessary, however, to establish and maintain the cells at maximum growth rates and utilize the cell at the early log phase.

Principles of Protoplast Culture:

The basic principle of protoplast culture is the aseptic isolation of large number of intact living protoplasts removing their cell wall and culture them on a suitable nutrient medium for their requisite growth and development. Protoplast can be isolated from varieties of plant tissues. Convenient and suitable materials are leaf mesophyll and cells from liquid suspension culture. Protoplast yield and viability are greatly influenced by the growing condition of the plant as well as the cells.

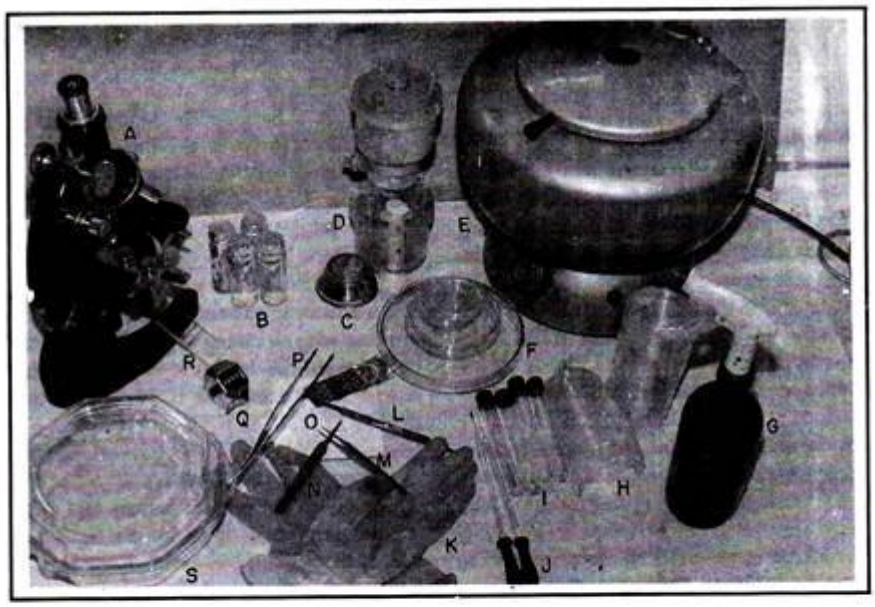


Fig 12.1

Instruments required for a plant protoplast culture. A. Compound microscope. B. Screw topped bottle. C. Nylon mesh. D. Bacterial filter. E. Centrifuge mechine. F. Petridishes. G. Alcohol sprayer. H. Disposable sterile petridishes. I. Screw capped centrifuge tube. J. Pasteur pipette. K. Hand gloves. L. Disposable sterile scalpel. M & N. Jewellery fine forceps. O. Tile. P. Long forceps. Q. Counter. R. Haemocytometer. S. Casserole. (Photograph taken by Mr. T. K. Bera)

The essential step of the isolation of protoplast is the removal of the cell wall without damaging the cell or protoplasts. The plant cell is an osmotic system. The cell wall exerts the inward pressure upon the enclosed protoplasts. Likewise, the protoplast also puts equal and opposite pressure upon the cell wall. Thus, both the pressures are balanced.

Now if the cell wall is removed, the balanced pressures will be disturbed. As a result, the outward pressure of protoplast will be greater and at the same time in absence of cell wall, irresistible expansion of protoplast takes place due to huge inflow of water from the external medium. Greater outward pressure and the expansion of protoplast cause it to burst.

So, the isolated protoplast is an osmotically fragile structure at its nascent stage. Therefore, if the cell wall is to be removed to isolate protoplast, the cell or tissue must be placed in a hypertonic solution of a metabolically inert sugar such as mannitol at higher concentration (13%) to plasmolysis the cell away from the cell wall (Fig 12.2).

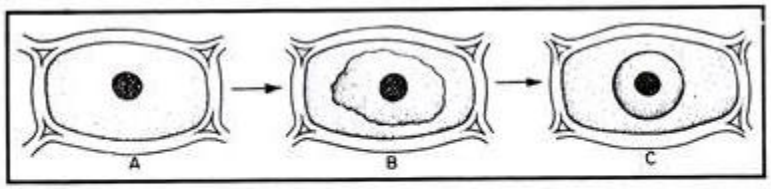


Fig 12.2

A – C. Showing the stages of plasmolysis. A. normal cell, B. Shrinking of protoplasm. C. complete plasmolysis

Mannitol, an alcoholic sugar, is easily transported across the plasmodesmata, provides a stable osmotic environment for the protoplasts and prevents the usual expansion and bursting of protoplast even after loss of cell wall. That is why, this hypertonic solution is known as osmotic stabilizer or plasmolyticum or osmolyticum.

Once the cells are stabilized in such a manner by plasmolysis the protoplasts are released from the containing cell wall either mechanically or enzymatically. Mechanical isolation (Fig 12.3) involves breaking open each cell compartment to liberate the protoplast. This operation can be done carefully on small pieces of tissue under a microscope using a micro-scalpel.

But very few protoplasts are obtained for a lot of time and effort. Large-scale attempts at mechanical isolation involves the disrupting tissue with fine stainless steel-bristled brush. This process may liberate more protoplasts with less efforts, but the percentage of yield of intact protoplasts is still very low.

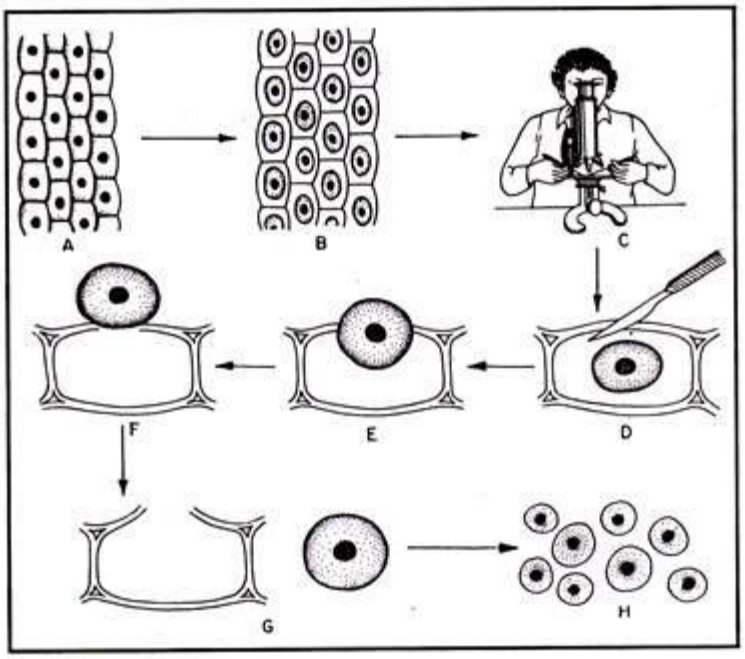


Fig 12.3

Method of mechanical isolation of protoplasts. A. A small piece of plant tissue. B. Plasmolysis of cells. C – D. Cutting of cell wall by microscalpel under microscope. E – F. Subsequent stages of liberation of protoplasts. G. Isolated protoplast and empty cell. H. Isolated protoplasts

A considerably more efficient way of liberating the protoplasts is to digest the cell walls away around them, using cell wall degrading enzymes such as cellulase, hemicellulose, pectinase or macerozyme etc. These enzymes are isolated from fungi and available commercially (Table 12.1).

Table 12.1 Commercial enzymes, their commercial name and source

Enzyme	Source organism
A. Cellulose degrading enzymes	
Cellulysin (Onozuka R10)	*Aspergillus niger*
Driselase	*Trichoderma reessei (formally T. viride)*
	Irpex lactes
B. Hemicellulose degrading enzymes	
Hemicellulase	*Aspergillus niger*
Rhozyme HP150	*A. niger*
C. Pectin degrading enzymes	
Pectinase	*A. niger*
Macerase (Macerozyme)	*Rhizopus spp.*
Pectinol AC, Pectolyase Y23	*A. niger*
Pectic-acid acetyl transferase (PATE)	*A. japonicus*

Period of treatment and concentration of enzymes are the critical factors and both factors should be standardized for particular plant tissue. Intact tissue can be incubated with a pectinase or macerozyme solution which will dissolve the middle lamella between the cells and so separate them.

Subsequent treatment with cellulase will digest away the cellulosic layer of the cell wall. This process is known as sequential enzyme treatment or two step method as opposed to a mixed enzyme treatment (one step method) in which both cellulase and pectinase or macerozyme are mixed so that the entire wall is broken down in a single operation (Fig. 12.4).

The isolated protoplasts can be cultured either static liquid or agarified medium. The protoplast media consist of mineral salts, vitamins, carbon sources and plant growth hormones as well as osmotic stabilizers and possibly organic nitrogen sources, coconut milk and organic acids.

In culture protoplast can reform a new cell wall around them. Once the wall is formed, the protoplast becomes a cell. The cells from protoplasts subsequently enter cell division which is followed by the formation of callus and cell cultures. Such callus also retain the capacity for morphogenesis and plant regeneration. A brief list of plant regeneration from plant protoplast culture is given below (Table 12.2).

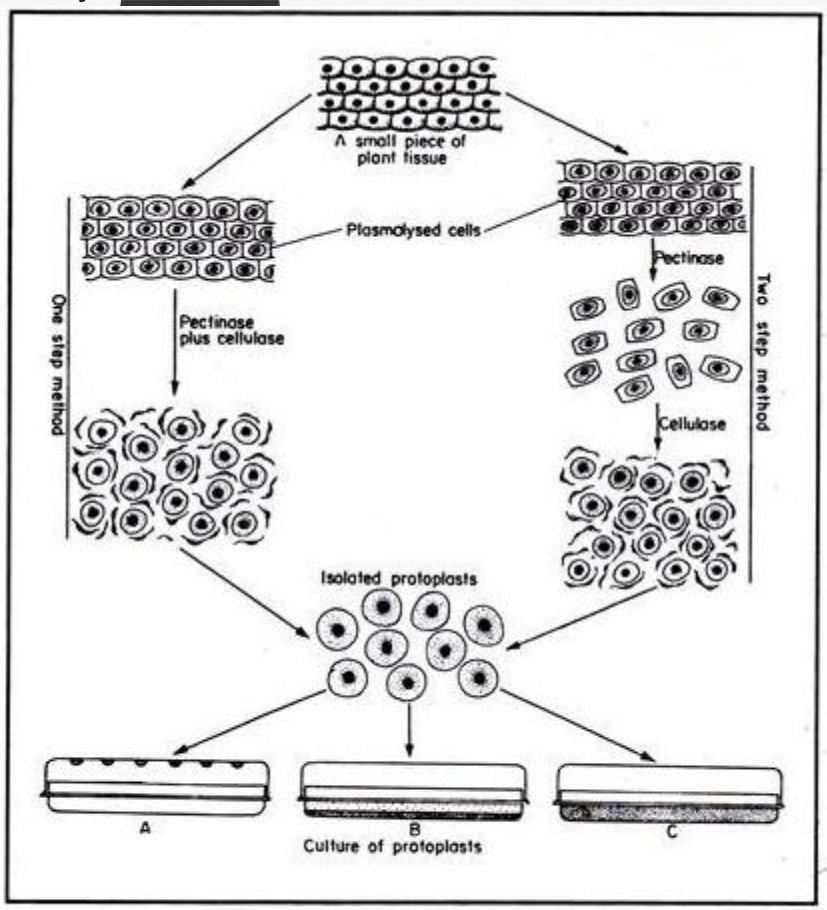


Fig 12.4

Methods of enzymatic isolation of a large number of protoplasts and their culture. A. Hanging-droplet method of culture. B. Co-culture. C. Plating of protoplasts

Table 12.2 Species in which plant regeneration has been achieved from cultured protoplasts

Common name	Species	Family	Cell origin
Tobacco	*Nicotiana tabacum*	Solanaceae	Leaf, cell culture
Potato	*Solanum tuberosum*	Solanaceae	Leaf
Datura	*Datura innoxia*	Solanaceae	Leaf
Petunia	*Petunia hybrida*	Solanaceae	Leaf
Carrot	*Daucus carota*	Umbelliferae	Cell culture
Rape seed	*Brassica napus*	Cruciferae	Leaf
Orange	*Citus sinensis*	Rutaceae	Nucellus callus
Asparagus	*Asparagus officinalis*	Liliaceae	Cladodes
Bromegrass	*Bromus inermis*	Poaceae	Cell culture

EMBRYOLOGY IN RELATION TO TAXONOMY- EGS. *TRAPA*, *EXOCARPUS*

Trapa was earlier included in Onagraceae family by Bentham and Hooker. But Engler separated it to family Trapaceae. It was supported by Hutchinson also. It has a different type of habit, heteromorphic leaves, swollen petiole, spiny fruit etc.

Embryological features like pyramidal pollen grains with 3 folded crests, semi inferior, bilocular ovary with single ovule in each locule, Polygonum type of embryosac, non-endospermic, fruit large one seeded drupe and one cotyledon extremely reduced as compared to Onagraceae where pollen grains are triangular, basin shaped, Superior, trilocular ovary with many ovules in each locule, Oenothera type embryosac, loculicidal capsule and both cotyledons of equal size.

Onagraceae and Trapaceae:

A monosporic tetranucleate embryo-sac is characteristic of all members of the Onagraceae and is not found in any other family of angiosperms. The genus *Trapa* having an eight-nucleate embryo-sac, which was once placed in the Onagraceae, has since been removed and assigned to a new family Trapaceae.

Manasi Ram's (1956) work on *Trapa bispinosa* fully confirms this view. Earlier, Eames (1953) expressed the view that on anatomical evidence also Trapa does not belong to the Onagraceae and is not even closely related to it. Table I presents the embryological differences between the families Onagraceae and Trapaceae.

Table I

	Trapaceae	Onagraceae
Ovary	Semi-inferior and bilocular with a single pendulous, anatropous, bitegmic ovule in each chamber.	Inferior, mostly tetralocular. Usually many anatropous bitegmic ovules in each chamber.
Megasporogenesis	The chalazal megaspore invariably functions and the embryo-sac is 8-nucleate	Usually the micropylar megaspore functions. The embryo-sac is tetranucleate
Endosperm	Endoserm lacking	Endosperm Nuclear
Proembryo	Solanad type	Onagrad type
Suspensor	Extremely well developed and haustorial	Short and inconspicuous haustorial
Embryo	Cotyledons extremely unequal	Cotyledons equal
Fruit	Large, one-seeded drupe	Usually a loculicidal capsule.

***Exocarpus*:**

Gagnepain & Boureau (1946, 1947), raised doubts about the position of *Exocarpus* and stated that instead of being regarded as an angiosperm it should be assigned to the gymnosperms on the basis of articulate pedicel, naked ovule and presence of pollen chamber and given a place somewhere near the Taxaceae. Lam (1948) commented as follows: **"At any rate, *Exocarpus* seems an interesting case and probably represents a transition between the protangiospermous gymnosperms and the Monochlamydeae".**

The embryological studies of Manasi Ram (1958) have clearly shown, however, that *Exocarpus* is a perfectly valid angiosperm with floral characters are similar to angiosperms, anther shows distinct endothecium and glandular tapetum, pollen grains have 2 cells at the time of shedding, an archesporial cell functioning as a megaspore mother cell, an embryo-sac of the Polygonum type, a cellular endosperm with a chalazal haustorium, and a pericarp derived from the wall of the ovary. Its correct position, therefore, lies in the Santalaceae to which it was assigned by previous systematists.