DSE 1
REPRODUCTIVE BIOLOGY OF ANGIOSPERM
UNIT 3 – ANTHER AND POLLEN **BIOLOGY**

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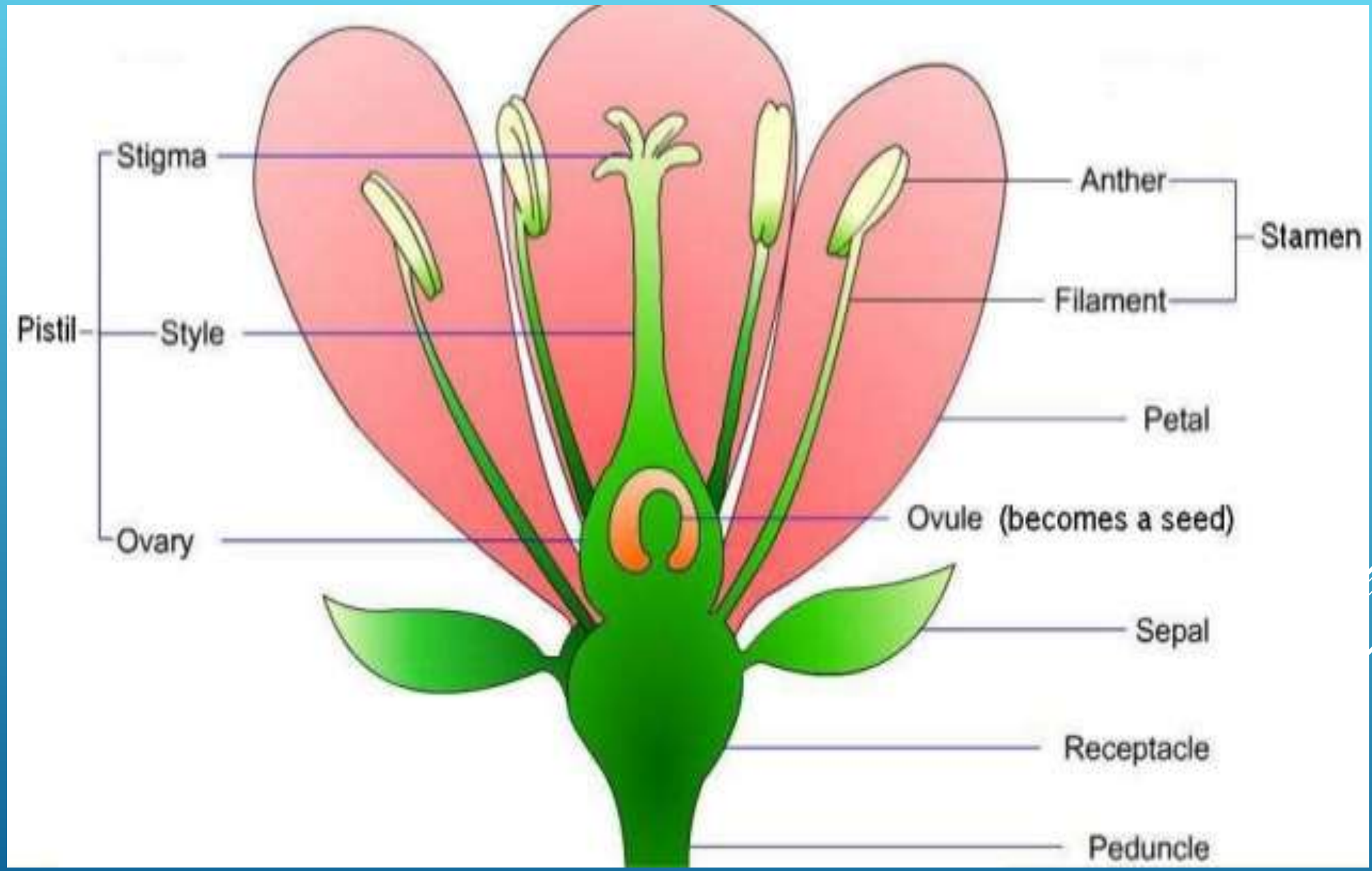
- **Anther wall :**
- **Structure & Functions,**
- **microsporogenesis, callose deposition and its significance.**
- **Microgametogenesis**

The Flower — What is it?

- thus, a flower is a specialized shoot that:

1. is determinate (vs. indeterminate)
2. has a modified stem with compressed internodes
3. possesses modified leaves with various functions, these determined by gene arrays (e.g., ABC model)
4. often clustered in an inflorescence (larger branch)


Stigma
Anther
Stamen
Filament
Pistil
Style
Petal
Ovule (becomes a seed)
Ovary
Sepal
Receptacle
Peduncle

Flowers

- Reproductive organs/structures of a plant
- Most flowers are **hermaphrodites** (contains both male and female sex organs)

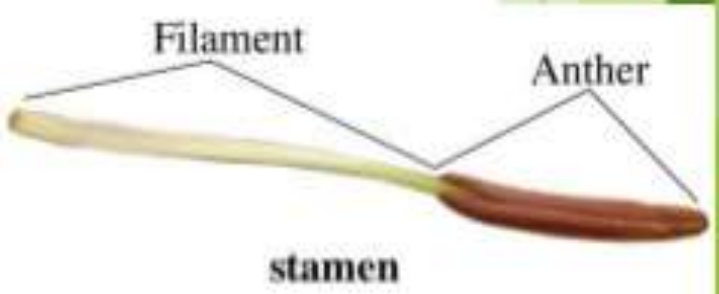
- In a flower, stamen is considered as the male reproductive organ. Each stamen consists of filament, connective and anther.
- Anther may be monothecous or dithecous. A monothecous anther consists of two locules or two sporangia. So it is said to be bilocular or bisporangiate.

- A dithecous anther consists of four locules or four sporangia. So it is said to be tetralocular or tetrasporangiate.
- Development of microsporangium is eusporangiate (= Sporangium developing from a group of cells).
- A very young anther in transverse section shows epidermis and archesporium.

Stamen

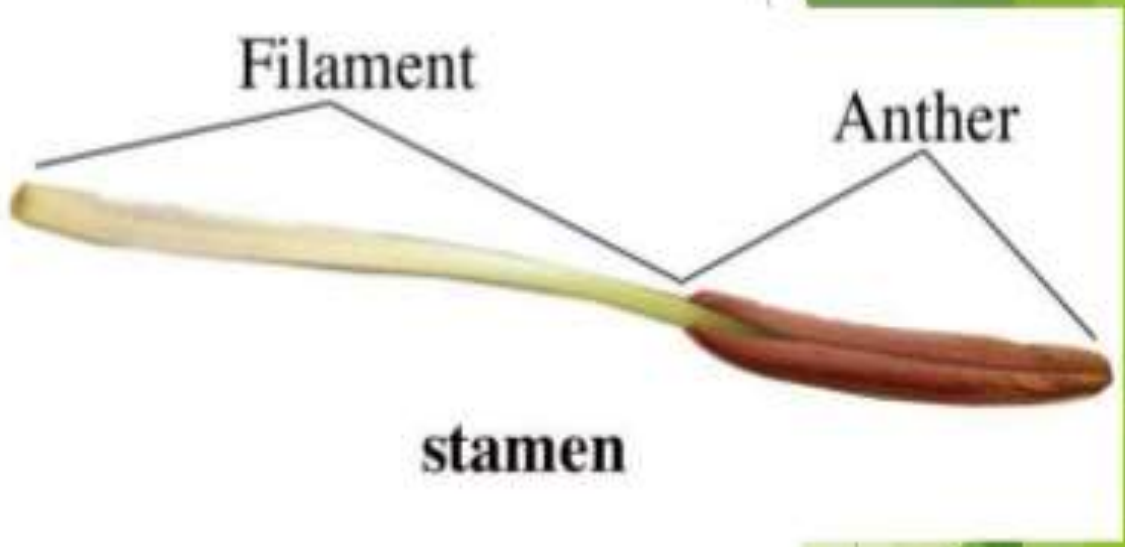
- The male reproductive organ
- Collectively called **androecium** (collective noun)
- Composed of two parts:

1. Anther
2. Filament



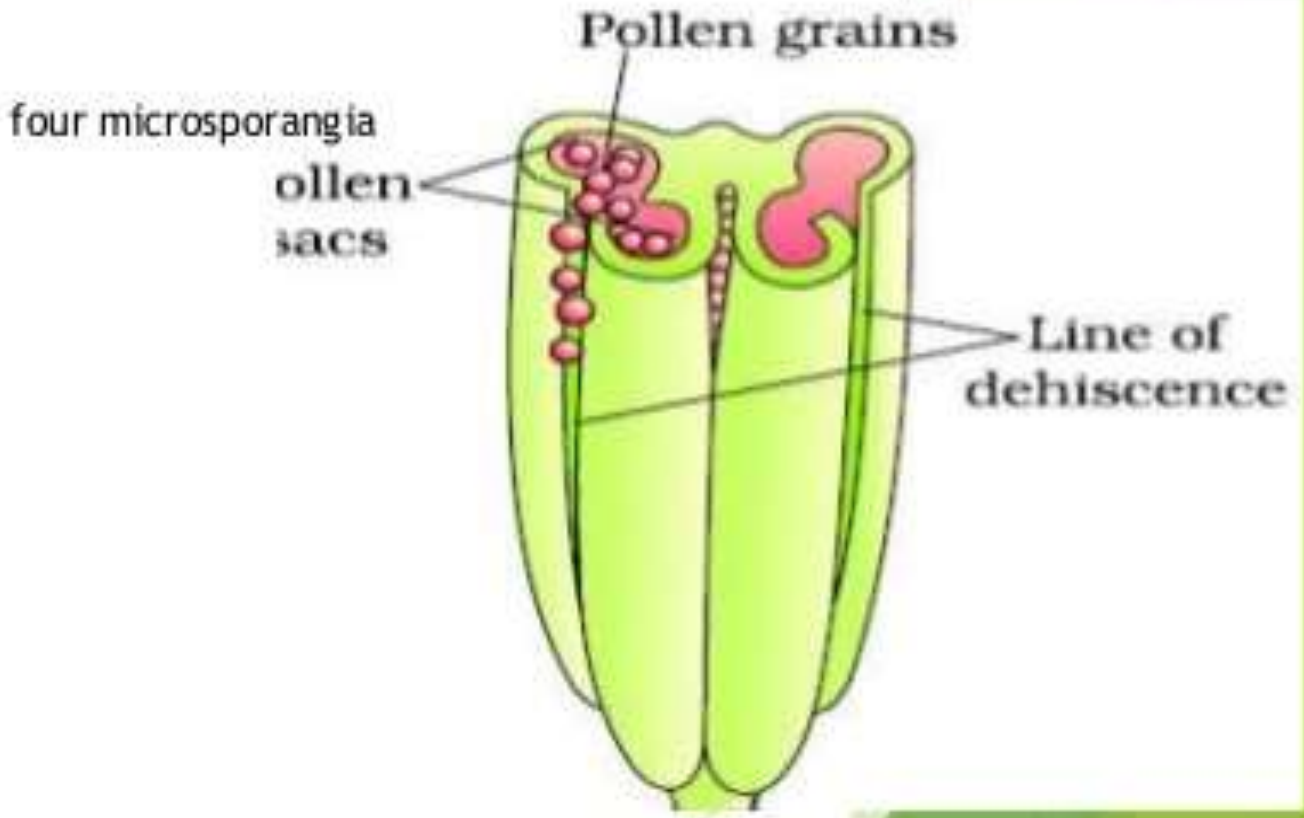
Anther

- Expanded lobular structure at the end of the filament
- Function: Produces **pollen** (male sex cell)



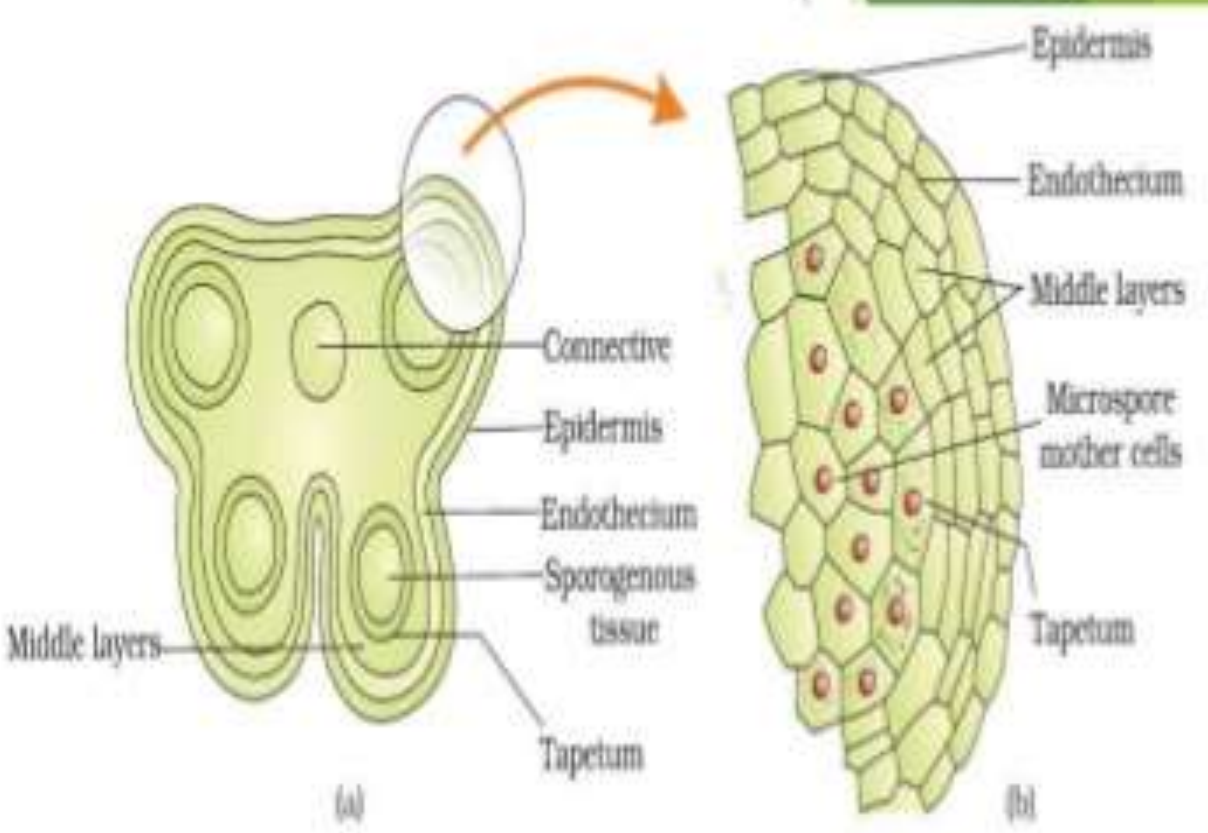
Anther: Structure

- Bilobed
- Dithecous
- longitudinal groove
- four-sided (tetragonal)
- four microsporangia (pollen sacs)



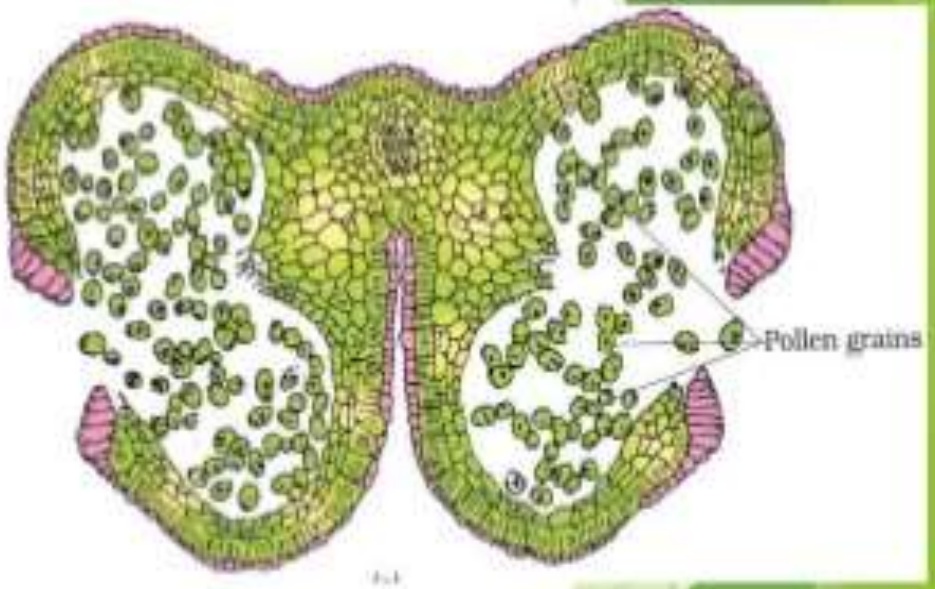
Anther wall layers

- Epidermis
- Endothecium
- Middle layers
- Tapetum
- Sporogenous tissue



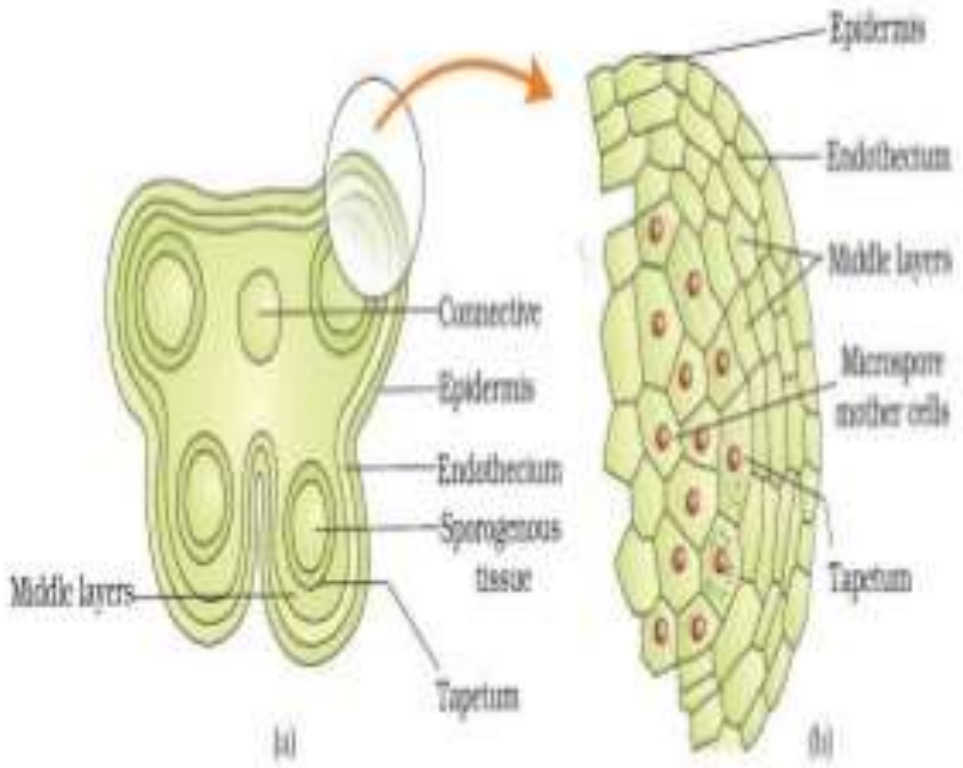
Anther wall layers

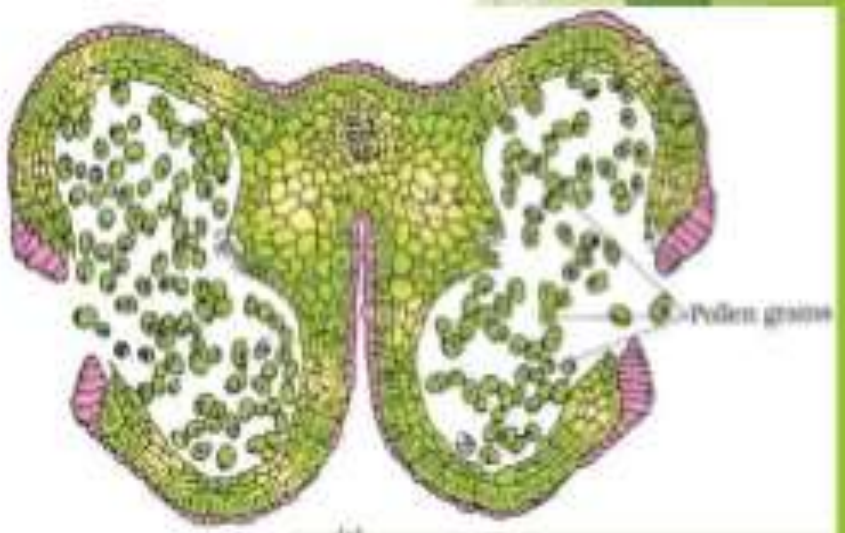
- **Epidermis:** one cell thick.
- **Function:** dehiscence of anther.
- **Stomium:** present between 2 pollen sacs.
- Anther dehiscence at this stomium region.



Anther wall layers

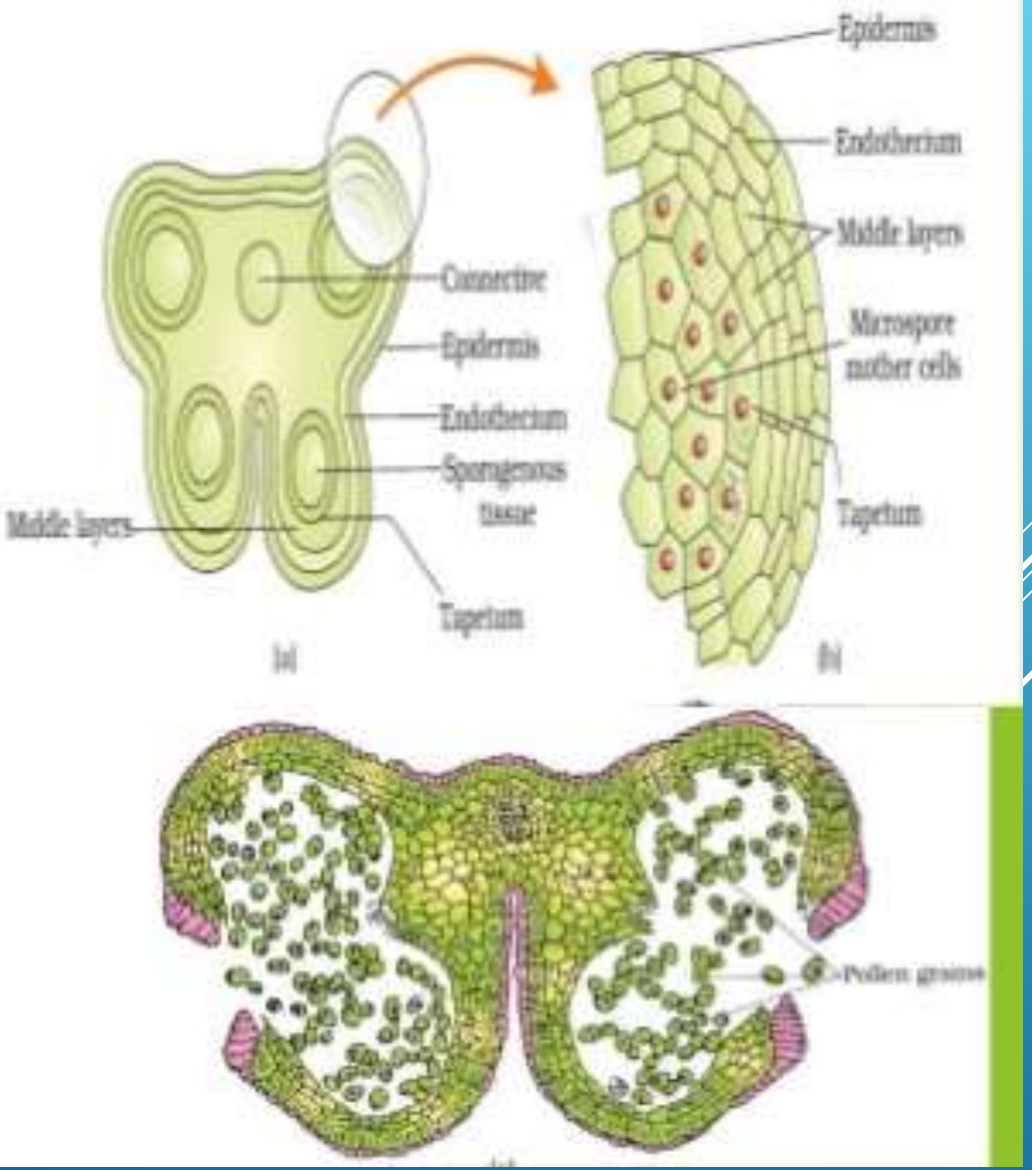
- **Endothecium**
- Below the epidermis.
- one cell thick.
- fibrous cellulose thickenings + little pectin.
- Function:at maturity these cells loose the water and helps in dehiscence of anther.





Anther wall layers

- **Middle layers:**
- **Below the endothecium.**
- **Function:at maturity these cells degenerate.**
- **helps in dehiscence of anther and protection of anther.**



The tapetum is a specialised layer of nutritive cells found between the sporogenous tissue and the anther wall within the anther of flowering plants. Tapetum is necessary for pollen grain nutrition and development, as well as a source of pollen coat precursors. The cells are often larger and feature more than one nucleus per cell. The nuclei of tapetal cells divide as sporogenous cells undergo mitosis. Because this mitosis is not always normal, many mature tapetum cells become multinucleate. Polyploidy and polyteny are also encountered on occasion. The tapetum's exceptionally large nuclear composition aids it in delivering nutrients and regulatory chemicals to the pollen grains as they develop.

Introduction

- Pollen grains, which contribute the male gametes, are formed within an anther.
- A typical anther is tetrasporangiate.

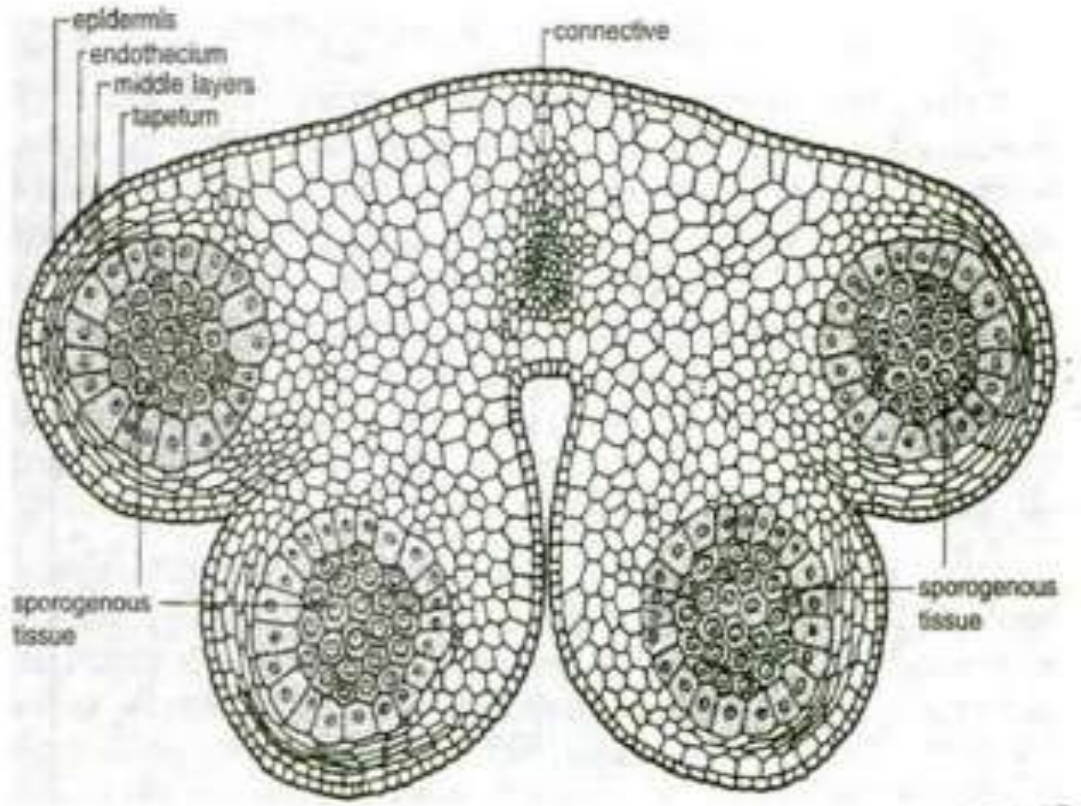
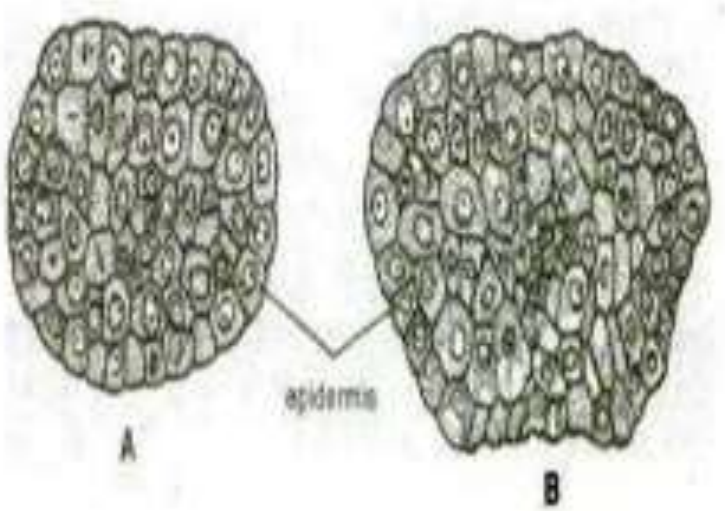
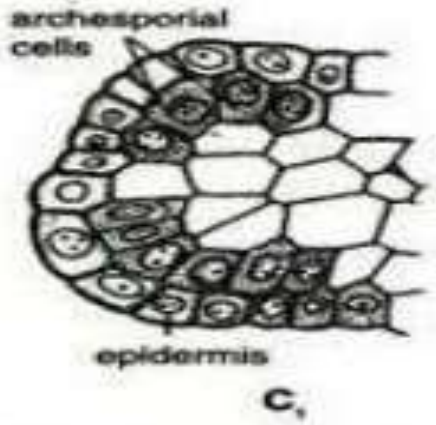


Fig. 3.1 Transverse section of a tetrasporangiate anther to show its various tissues.

Anther Wall Development

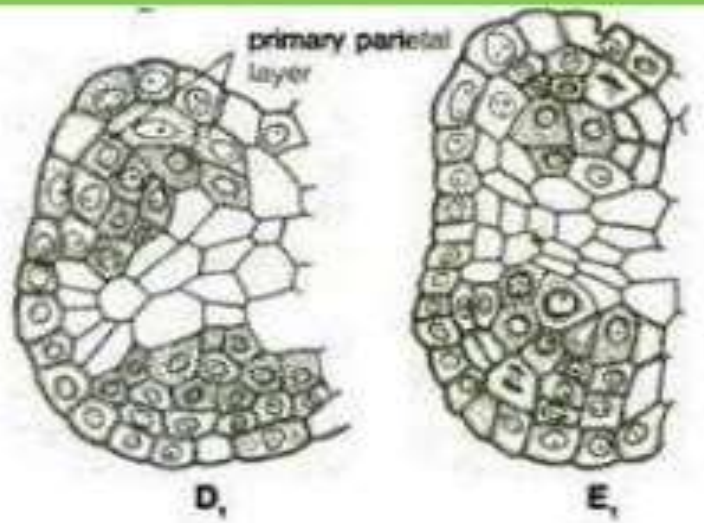
- A Very young anther comprises a homogeneous mass of cells bounded by a well defined epidermis.
- During its development the anther assumes a four lobed appearance.
- In each lobe some hypodermal cells become more prominent – these cells constitute the archesporium.

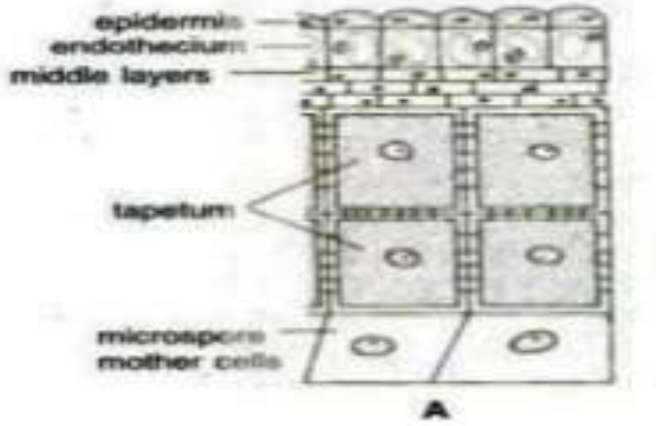




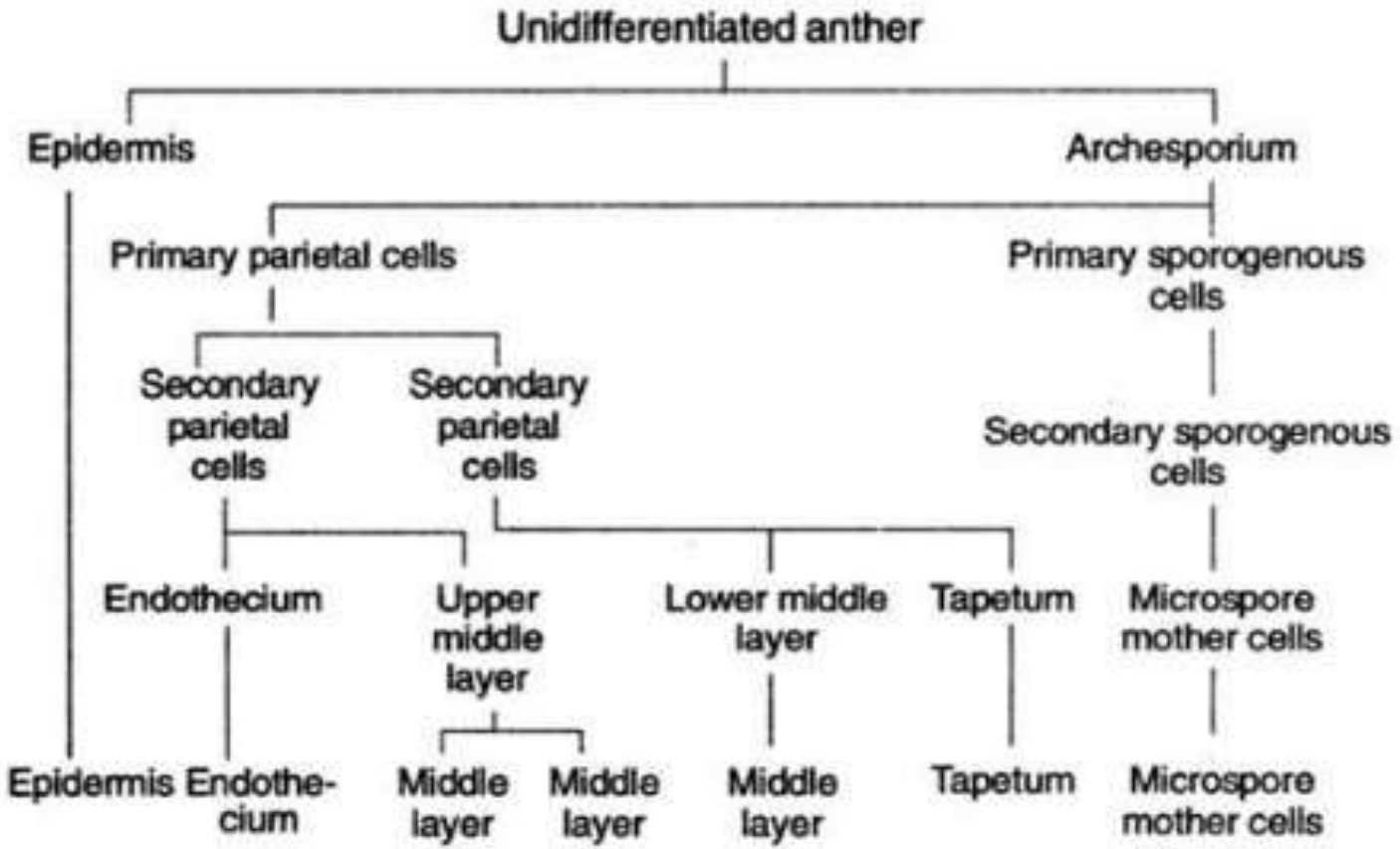
Anther Wall Development

- The archesporial cells divide into periclinal division .
- Primary parietal cells towards the epidermis.
- Primary sporogenenous cells toward the interior of the anther.
- The cells of the parietal layer undergo a series of periclinal & anticlinal divisions – 2 to 5 concentric layers of anther wall.
- The primary sporogenous cells, either directly or after a few mitoses, function as microspore mother cell.



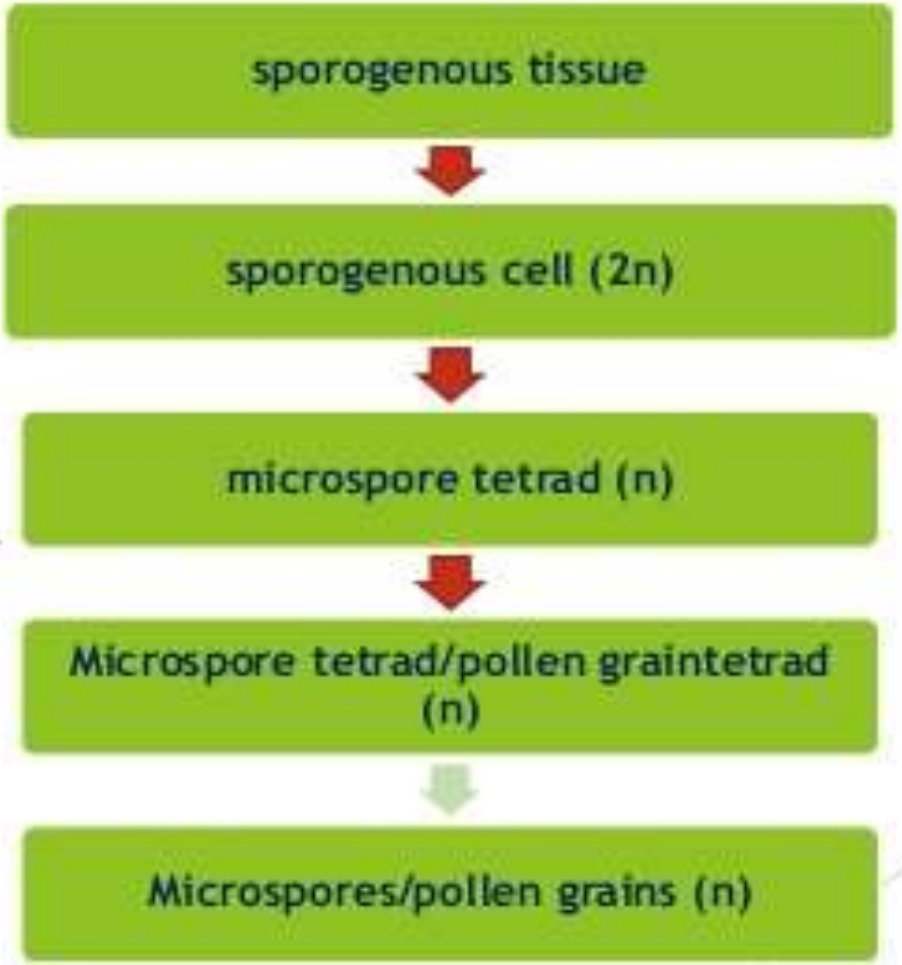


- The archesporial cells divide periclinally giving rise to primary parietal cells on the outer side and sporogenous cells towards inner side.
- The cells of the parietal layer divide periclinally and anticlinally forms endothecium, middle layers and tapetum.
- The cells of the primary sporogenous tissue differentiated into pollen mother cells or microspore mother cells.

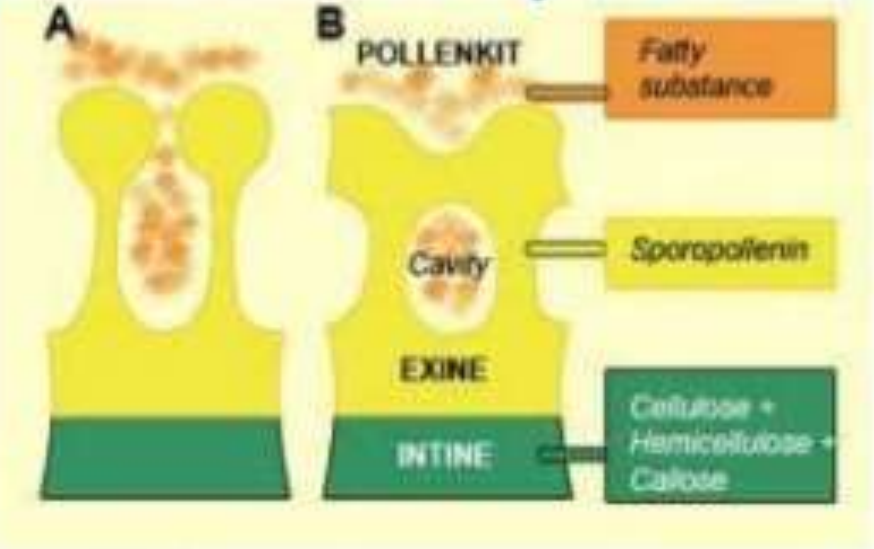

Unidifferentiated anther
Epidermis
Archesporium
Primary parietal cells
Primary sporogenous cells
Secondary parietal cells
Secondary parietal cells
Secondary sporogenous cells
Endothecium
Upper middle layer
Lower middle layer
Tapetum
Microspore mother cells
Epidermis
Endothe-cium
Middle layer
Middle layer
Middle layer
Tapetum
Microspore mother cells

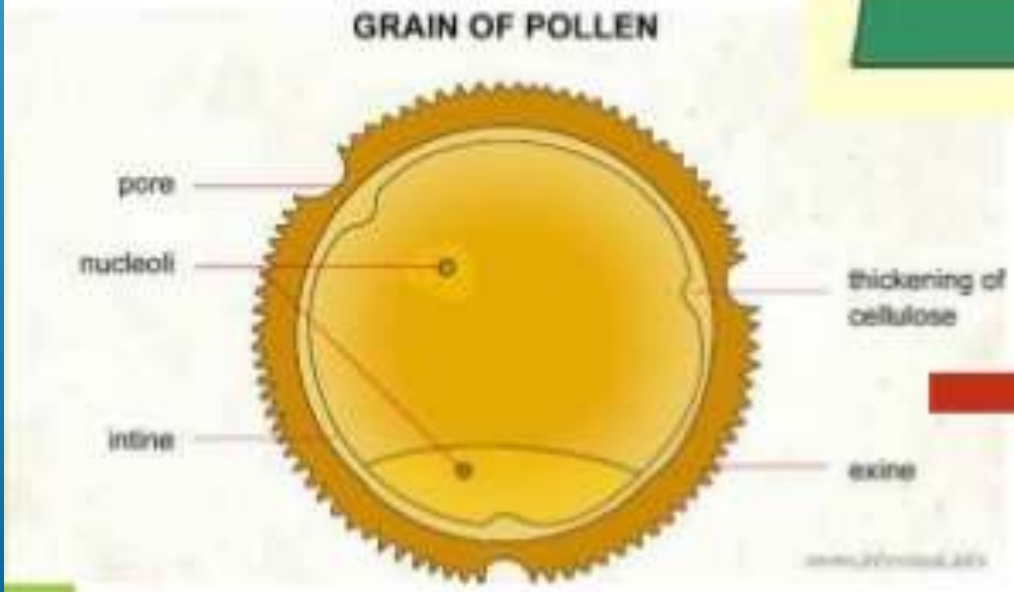
Microsporogenesis

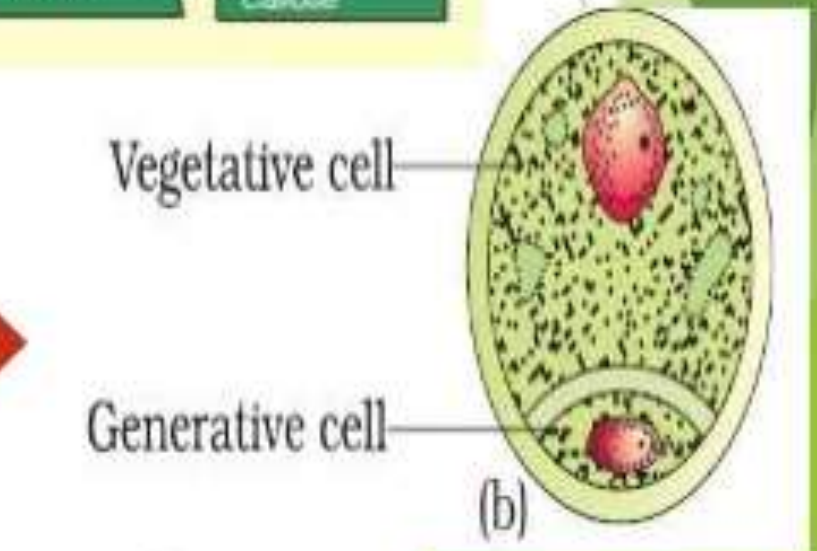
Formation and differentiation of microspore is called microsporogenesis.



Structure of Pollen/microspore







DEVELOPMENT OF MICROSPORES IN ANTHER

- Microspores i.e., the pollen grains, are developed inside microsporangia. The microsporangia are developed inside the corners of the 4-lobed anther.
- Young anthers are more or less oblong in shape in section and made up of homogeneous mass of meristematic cells without intercellular space. With further development, the anther becomes 4-lobed. The outer layer of anther is called epidermis.
- Below the epidermis, at each corner, some cells become differentiated from others by their dense protoplasm — 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.

- The outer primary parietal cells form primary parietal cell layer at each corner. 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.
- The innermost layer of antheridial wall, which remains in close contact with the sporogenous tissue, functions as nutritive layer, called tapetum.

SPOROGENOUS TISSUE AND ANTHEREDIAL WALL

- 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. The spore mother cell undergoes meiotic division and gives rise to 4 microspores arranged tetrahedrally.

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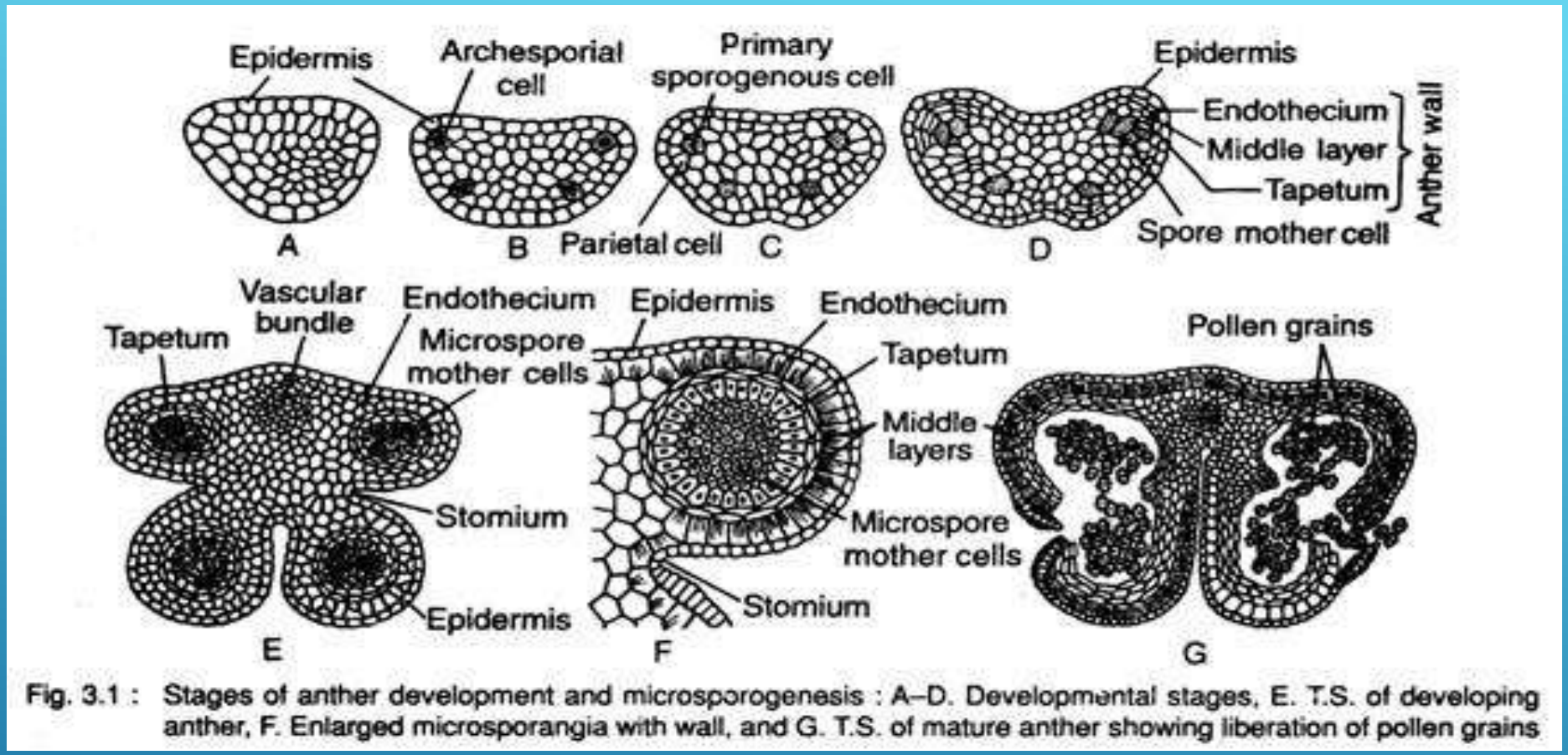

Fig. 3.1 : Stages of anther development and microsporogenesis : A–D. Developmental stages, E. T.S. of developing anther, F. Enlarged microsporangia with wall, and G. T.S. of mature anther showing liberation of pollen grains

STAGES OF ANTHER DEVELOPMENT AND MICROSPOROGENESIS

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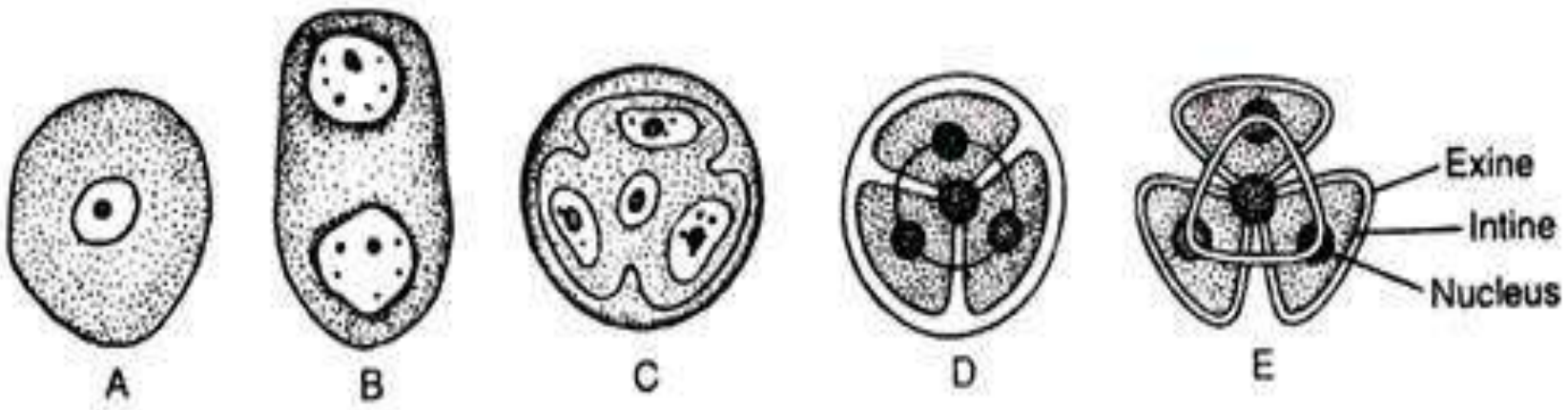

Fig. 3.2 : Different stages of development of microspore from microspore mother cell : A. Microspore mother cell, B. Diad stage, C. Tetrad stage, D. Clevage of protoplast and formation of pollen grains, and E. Four microspores i.e., pollen grains with exine and intine

STAGES OF DEVELOPMENT OF MICROSPORE FROM MICROSPORE MOTHER CELL

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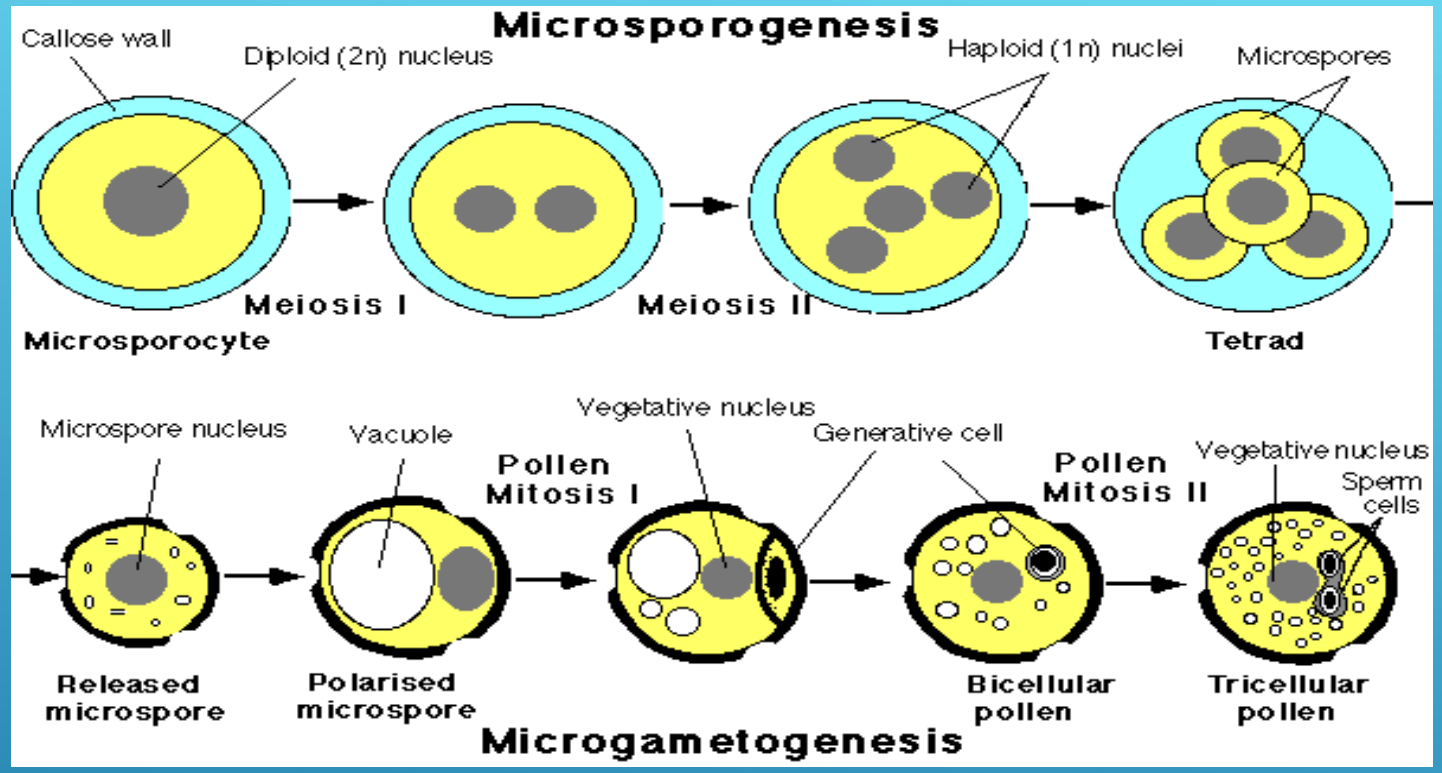
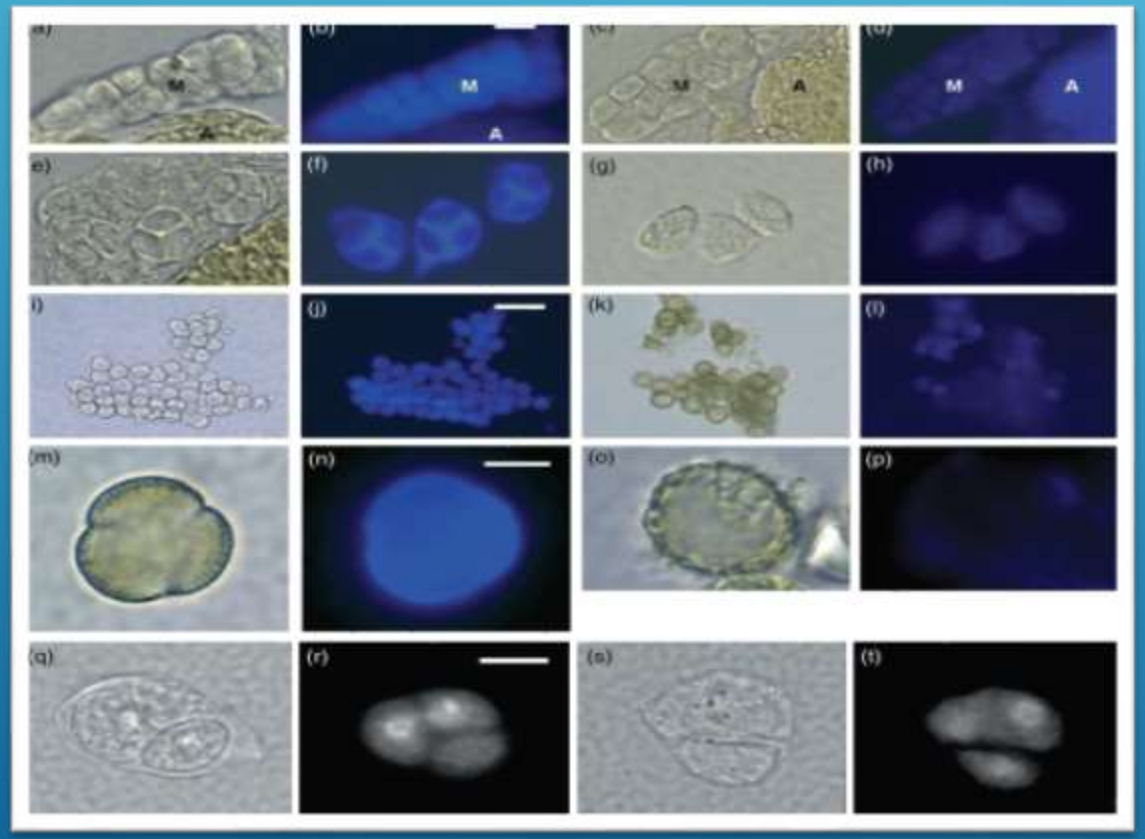


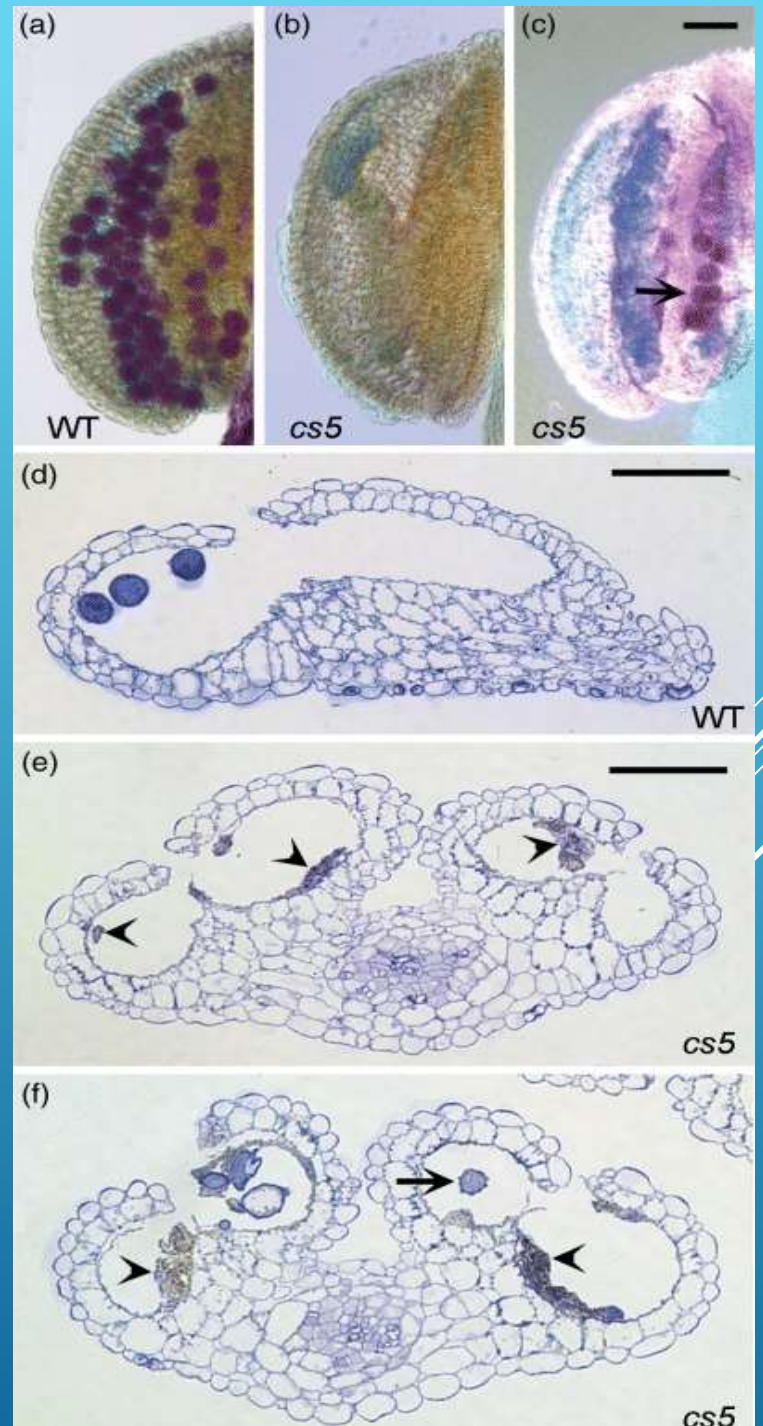
CALLOSE IS A COMPONENT OF CELL WALLS IN HIGHER PLANTS. ALTHOUGH IT IS NOT AS COMMON AS CELLULOSE, ITS ROLE IS VERY SIGNIFICANT. IT GENERALLY EXISTS IN SMALL QUANTITIES IN STRUCTURALLY DIFFERENT PLANT TISSUES, AND IT HAS INDIVIDUAL PROPERTIES: (1) HIGH IMPERMEABILITY, (2) RAPID SYNTHESIS AND EASY DEGRADATION. CALLOSE CAN BE IDENTIFIED WITH ANILINE BLUE BY FLUORESCENCE MICROSCOPE

https://www.researchgate.net/profile/zonglie_hong

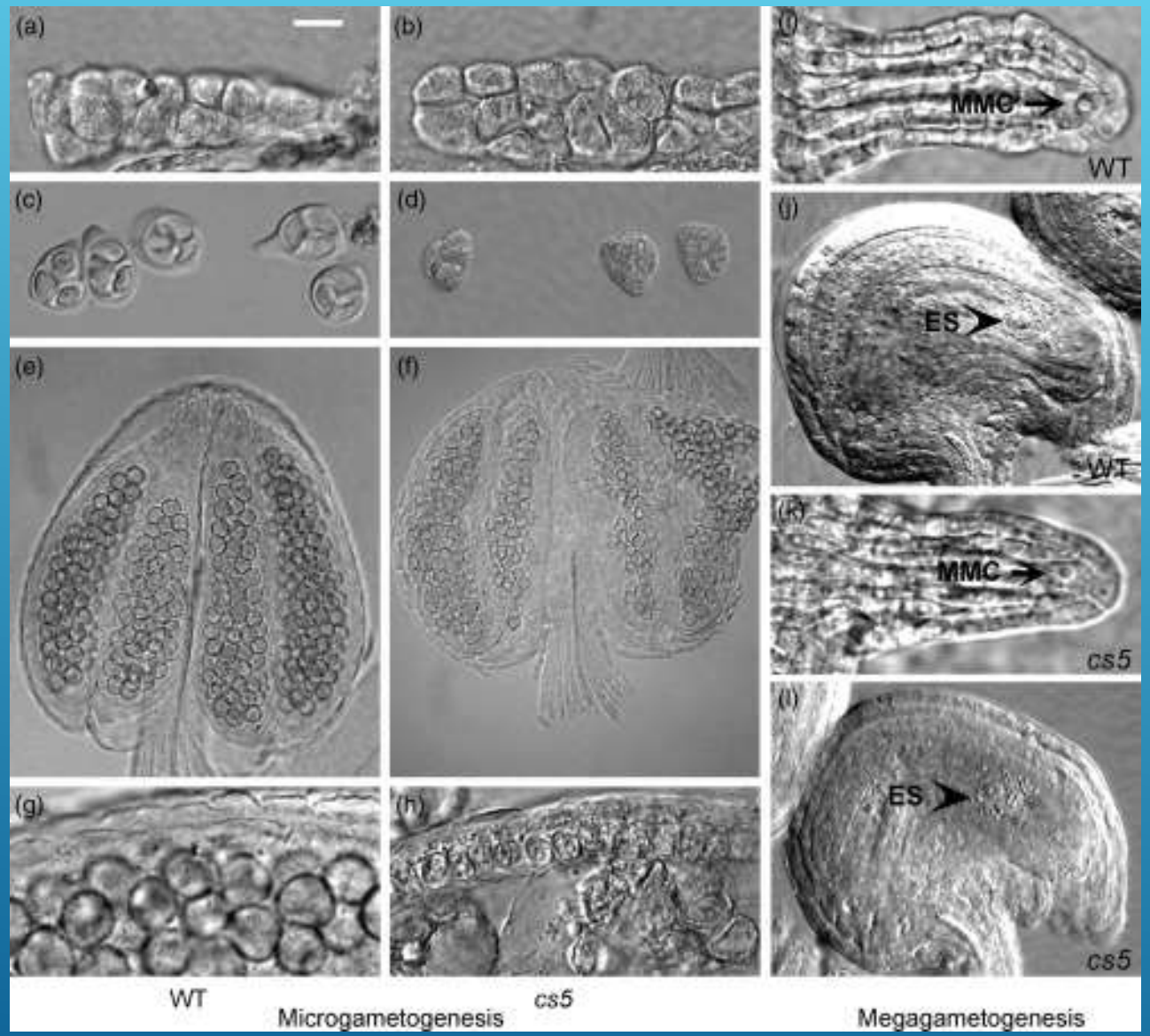
CALLOSE DEPOSITION DURING MICROSPOROGENESIS. NOTE THAT CALLOSE DEPOSITION WAS NEARLY ABSENT IN MEIOCYTES, TETRADS, MICROSPORES, AND POLLEN GRAINS IN THE MUTANT PLANTS. (B, E) BUT PRESENT IN MEIOCYTES OF WILD TYPE (A,D)

ANTHER OF THE WILD-TYPE PLANTS IS FILLED WITH VIABLE, PURPLE-STAINED POLLEN GRAINS (PURPLE; A), WHILE THAT OF THE MUTANTS CONTAINS ONLY NON-VIABLE POLLEN GRAINS (GREEN; B) OR ONLY A FEW VIABLE POLLEN GRAINS (ARROW; C).





CALLOSE SYNTHESIS FOR EXINE FORMATION AND POLLEN VIABILITY



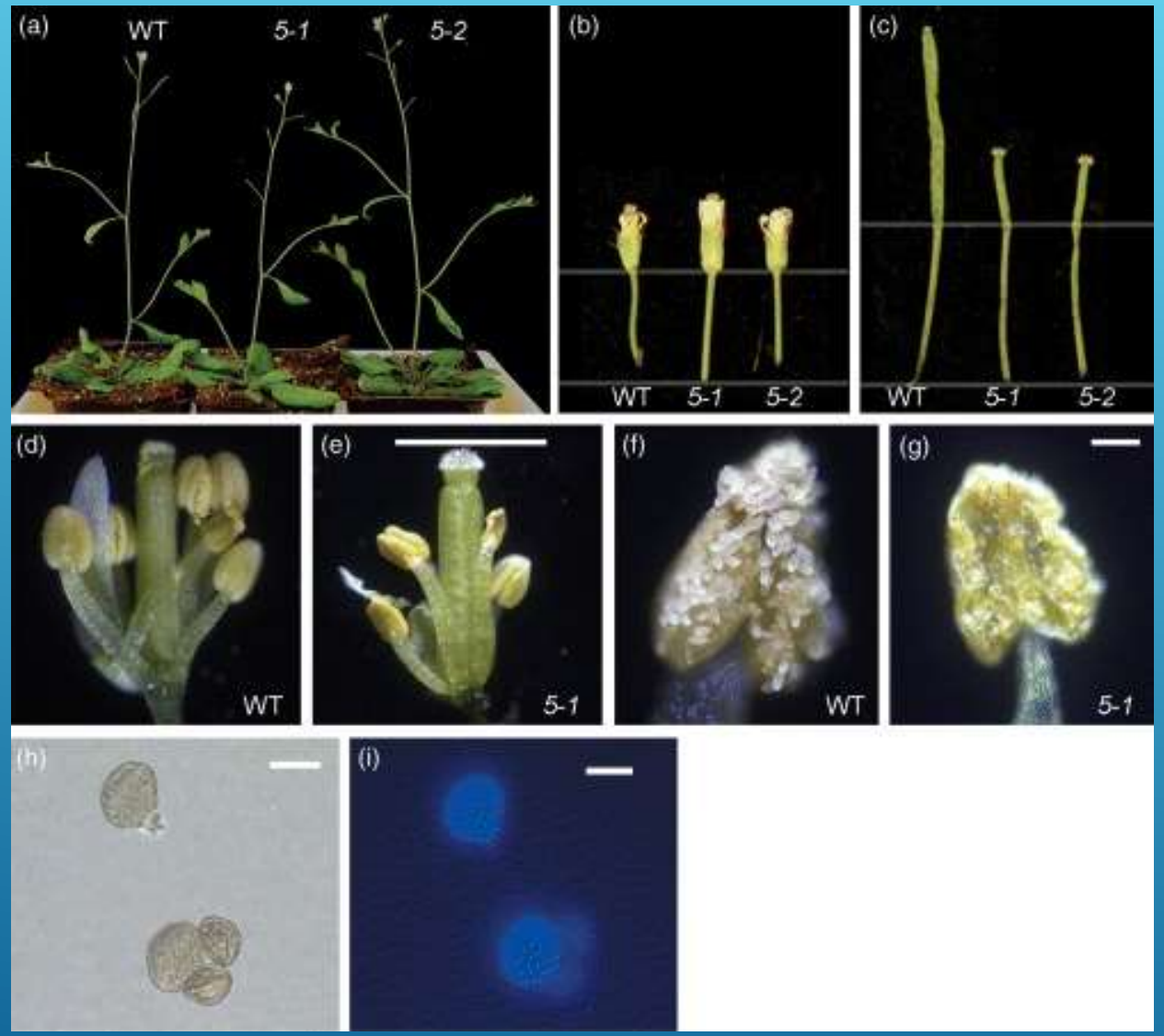
(a, b) Developing pollen mother cells shows uniform meiocytes in the wild type (a) and mutant plants (b).

(c, d)The microspores of wild-type plants are encased in callose walls and are separated by clear boundaries. In the mutants, the four microspores form a clump and the boundaries are barely recognizable.

(e, f) Comparison of microspores →After the release from the tetrad, microspores continue to develop into round and mature pollen grains in the wild-type plants, whereas they start to deform and degenerate in the mutants. Bar = 50 μm.

(g, h) Enlarged views of the developed microspores in wild-type plants and the degenerated microspores in the mutants. Bar = 50 μm.

(i–l) Megaspore mother cell (MMC, arrow) of the wild type (i) and mutant (k), and embryo sac (ES, arrowhead) of the wild type (j) and mutant (l). Note that there are no apparent differences in embryo sac development between the wild type and mutant plants

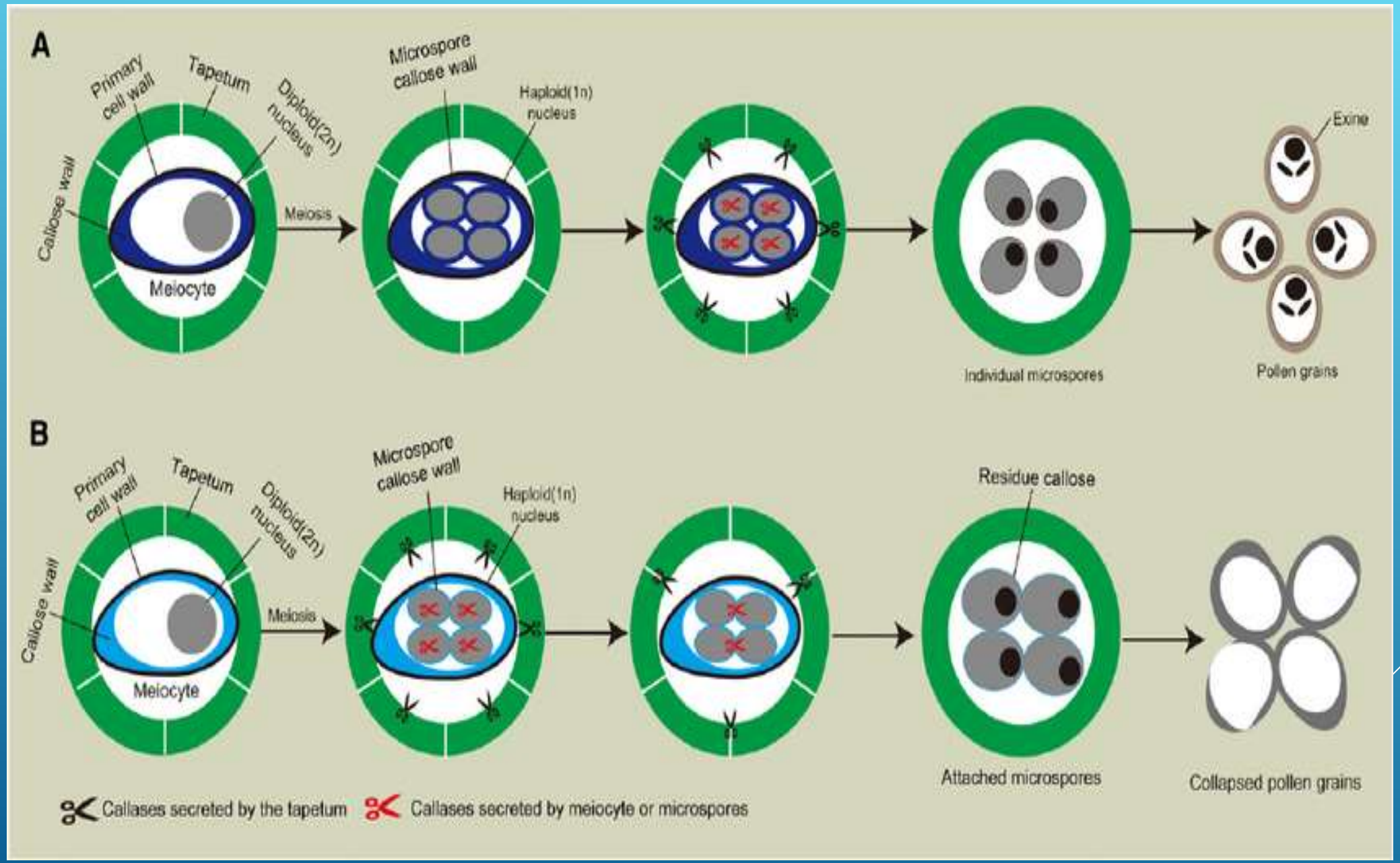


REDUCTION IN FERTILITY OF FLOWER

Anthers are shrunken in mutant type where pollens are non-viable due to lack of deposition of callose wall.

(d) Flower-normal

(e) Flower – not fertile


A
Primary cell wall
Tapetum
Diploid(2n) nucleus
Callose wall
Meiocyte
Meiosis
Microspore callose wall
Haploid(1n) nucleus
Individual microspores
Exine
Pollen grains
B
Primary cell wall
Tapetum
Diploid(2n) nucleus
Callose wall
Meiocyte
Meiosis
Microspore callose wall
Haploid(1n) nucleus
Residue callose
Attached microspores
Collapsed pollen grains
Callases secreted by the tapetum
Callases secreted by meiocyte or microspores

CALLOSE DEPOSITION

- In developing anthers of angiosperms, microsporocytes synthesize a specialized temporary cell wall consisting of callose (a b-1,3-linked glucan) between the primary cell wall and the plasma membrane. Callose continues to be deposited through both meiotic divisions and it eventually encloses the microspore tetrad in a thick callose wall.
- Callose synthesis has a vital function in building a properly sculpted exine, the integrity of which is essential for pollen viability.
- The callose wall may also function as a ‘molecular filter’ protecting the developing microspores from the influence of the surrounding diploid tissues
- It also provides a physical barrier that may help prevent premature swelling and bursting of the microspores.
- Callase activity in the anthers is low during the first meiotic division but increases rapidly at the end of the second meiotic division. Finally, the callose wall is degraded and microspores are released in the locular space

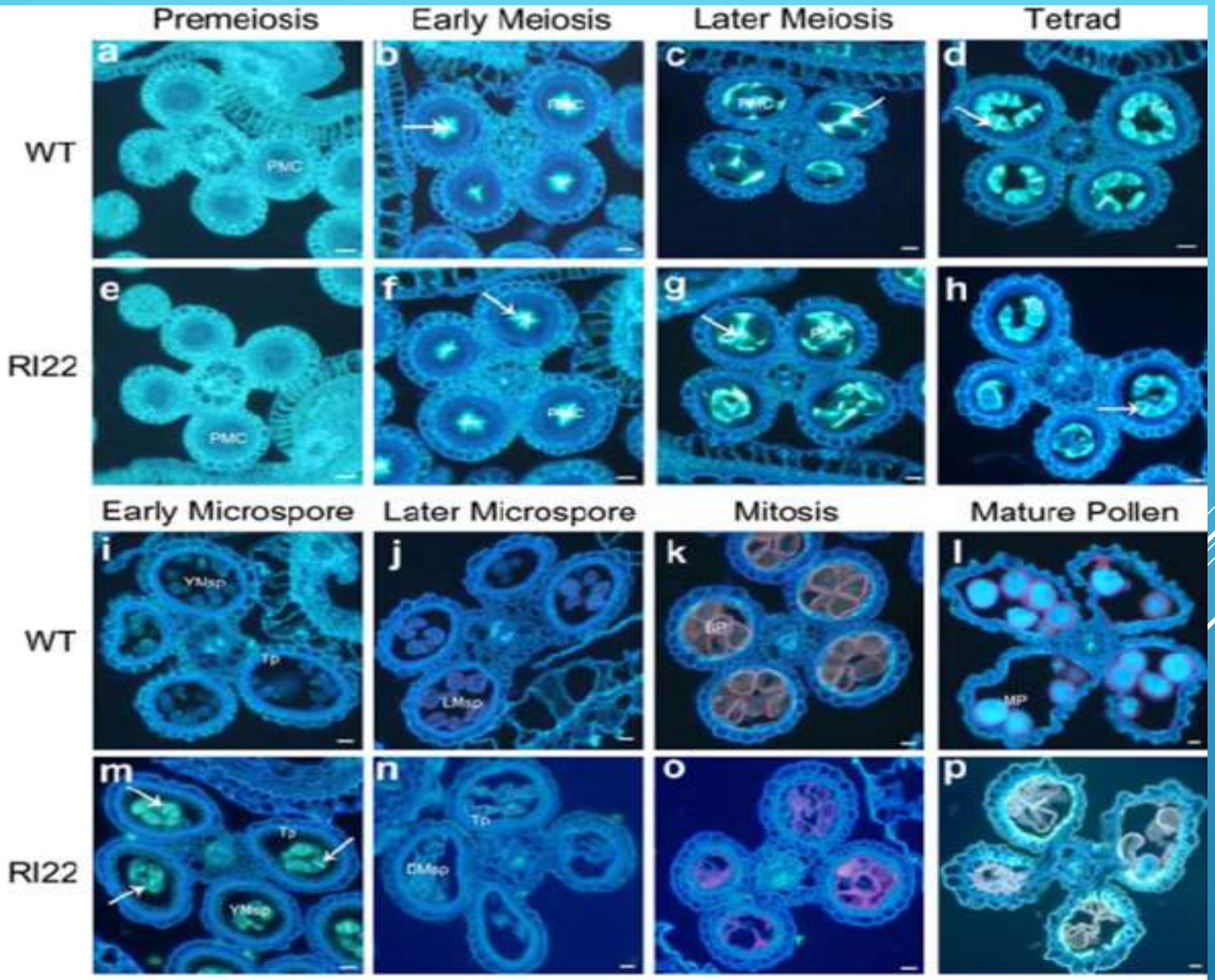
CALLOSE DEPOSITION

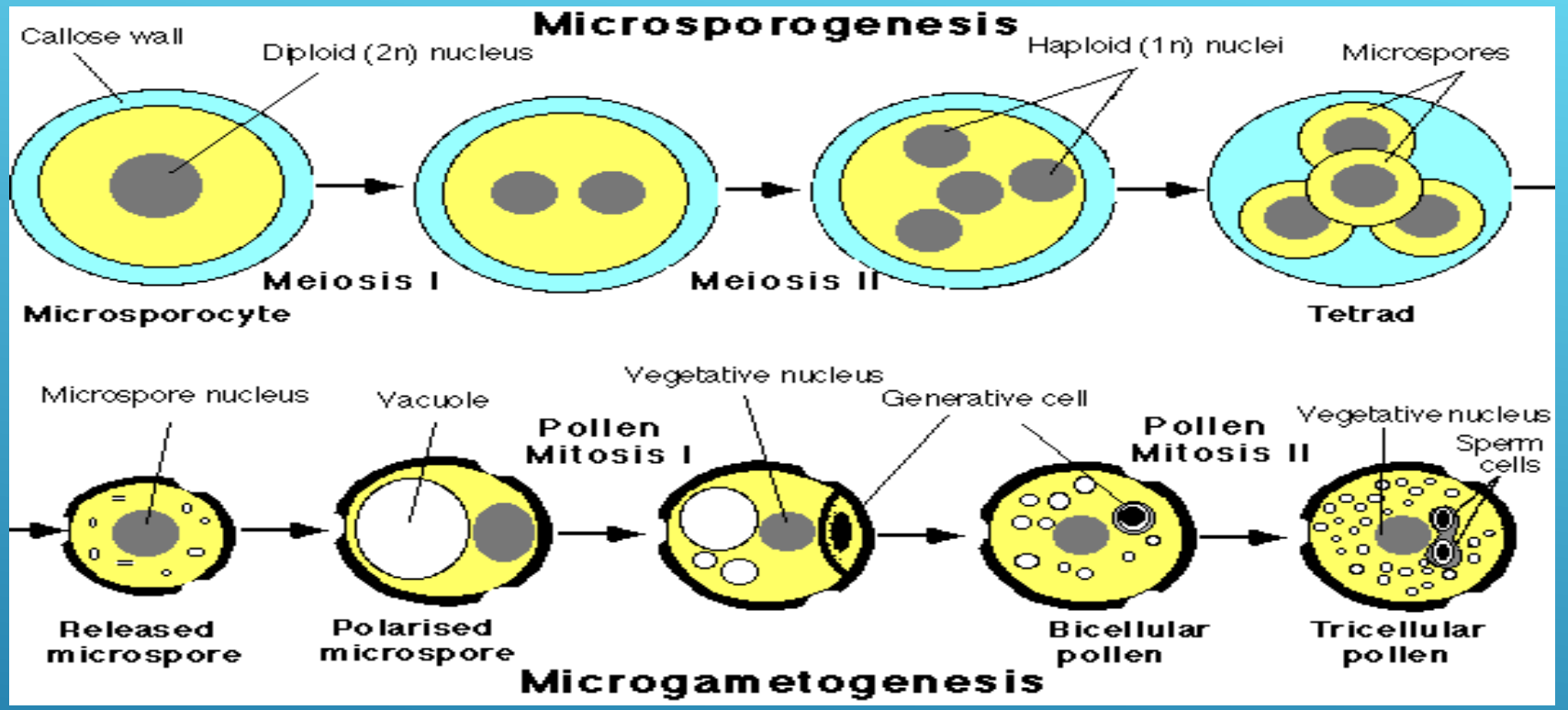
- Callose is a β-1, 3-linked homo polymer of glucose that contains some 1,6 branches. It is widespread in multicellular green algae and higher plants, including monocots and dicots, and is an essential component of specialized cell walls or cell wall-associated structures at various stages of plant development
- Callose walls, temporary cell walls consisting of callose deposited between the primary cell wall and the plasma membrane, may function in the maintenance of microsporocyte morphology and to shield microsporocytes from the influence of the surrounding environment.
- Callose walls prevent microsporocytes fusion and cohesion, and the timely degradation of the walls facilitates the release of microspores from tetrads during late meiosis. Temporary callose walls act as a physical barrier to prevent premature swelling and bursting of microspores; moreover, they appear to participate in the formation of the primexine by providing a mold for pollen exine construction during microsporogenesis.
- Further, the pollen tube, which functions in the transport of sperm to female gametes, contains callose both in their walls and in the plugs. The cell plate is also mainly composed of callose; callose deposition at the cell plate is tightly linked to the depolymerization of microtubules. Thus, callose plays an essential role in meiosis.

CALLOSE PLAYS IMPORTANT ROLES

- It plays a significant role in the reproductive biology of angiosperms, particularly.
- Callose wall surrounds the sporocytes while meiosis occurs.
- Because of its structure, it may provide an isolation barrier sealing off one meiotic cell (pollen mother cell or megaspore mother cell) from another .
- It acts as a temporary wall to prevent the products of meiosis from cohesion and fusion, and its dissolution results in the release of free spores.
- It has been proposed that the callose wall functions as a molecular filter isolating the developing microspores from the influence of the surrounding diploid tissue or sister spores . The molecular filter also transmits only signals that are indispensable for meiosis into the meiocytes

Fig. 5 Callose deposition in anthers during male gametogenesis. Cross-sections of wild-type and *Osg1-RI* (RI22) plants anthers were stained with aniline blue to detect callose. Wild-type sections are shown in **a**–**d** and **i**–**l**, and other *panels* show *Osg1-RI* plant sections. Callose deposition is shown as *bright-yellow* fluorescence (indicated by *arrows*). Note that the callose signal was still strong around the pollen of *Osg1-RI* plants at the early microspore stage. *Tp* tapetum layer, *PMC* pollen mother cell, *Td* tetrad, *YMsp* young microspore, *LMsp* later microspore, *DMsp* degenerated microspore, *BP* binucleate, *MP* mature pollen, *RI22* RNAi plant of line 22. *Bars* 10 μm. Anthers at the premeiosis (**a**, **e**), early meiosis (**b**, **f**), later meiosis (**c**, **g**), tetrad (**d**, **h**), early microspore (**i**, **m**), later microspore (**j**, **n**), pollen mitosis (**k**, **o**), and mature pollen stages (**l**, **p**)





Gametogenesis is a biological process by which haploid male and female gametes are formed. In higher plants, there are two stages that are involved- sporogenesis and gametogenesis. Sporogenesis is the formation of spores whereas gametogenesis is the formation of gametes

https://www2.le.ac.uk/departments/genetics/people/twell/lab/images/dev.gif

- Microspore i.e., the pollen grain, is the first cell of the male gametophyte, which contains only one haploid nucleus.
- The pollen grains have two walls — outer exine (the exine is further differentiated into two regions, outer sexine and inner nexine) and inner intine.

SPOROGENSIS AND GAMETOGENESIS

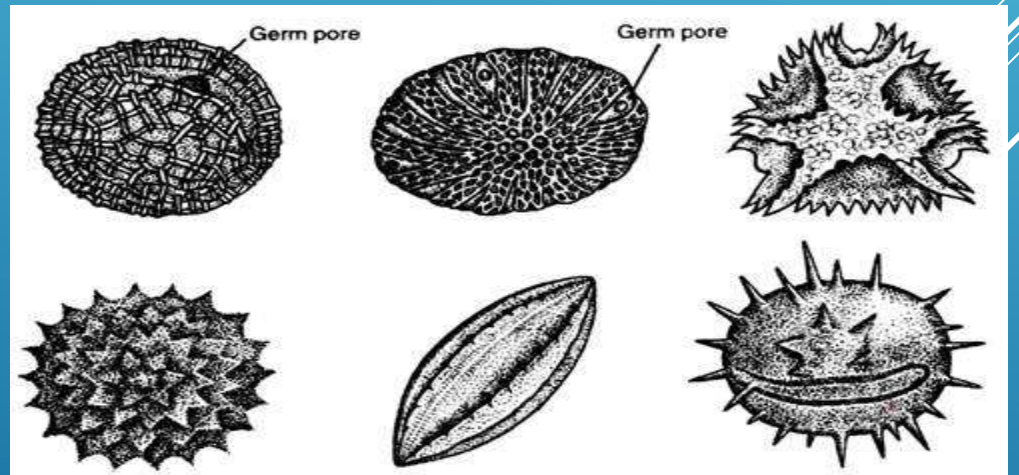
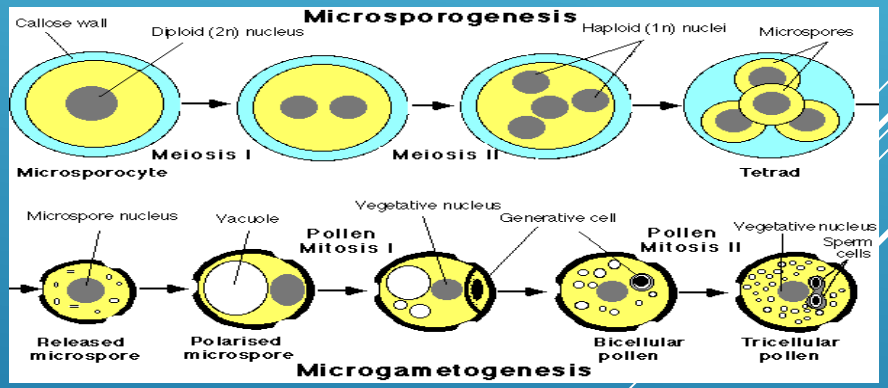


Fig. 3.3 : Different pollen grains showing various types of sculpturing

- During early stage of development, it remains within the microsporangium. The cell undergoes unequal division and forms a small generative cell and a large vegetative or tube cell. Initially the generative cell remains lying at one corner of the spore wall.
- Within short time, it gets detached and becomes ellipsoid or fusiform in shape and remains suspended in the cytoplasm of the vegetative cell (2-celled stage i.e., vegetative cell and generative cell). Later on, the generative cell divides and gives rise to two ellipsoidal or lenticular or spherical cells — the male gametes (3-celled stage i.e., vegetative cell and two male gametes



MICROGAMETOGENESIS OR DEVELOPMENT OF MALE GAMETOPHYTE

MICROGAMETOGENESIS

- is the process in plant reproduction where a microgametophyte develops in a pollen grain to the three-celled stage of its development. In flowering plants it occurs with a microspore mother cell inside the anther of the plant.
- When the microgametophyte is first formed inside the pollen grain four sets of fertile cells called sporogenous cells are apparent. These cells are surrounded by a wall of sterile cells called the tapetum, which supplies food to the cell and eventually becomes the cell wall for the pollen grain. These sets of sporogenous cells eventually develop into diploid microspore mother cells. These microspore mother cells, also called microsporocytes, then undergo meiosis and become four microspore haploid cells. These new microspore cells then undergo mitosis and form a tube cell and a generative cell. The generative cell then undergoes mitosis one more time to form two male gametes, also called sperm.

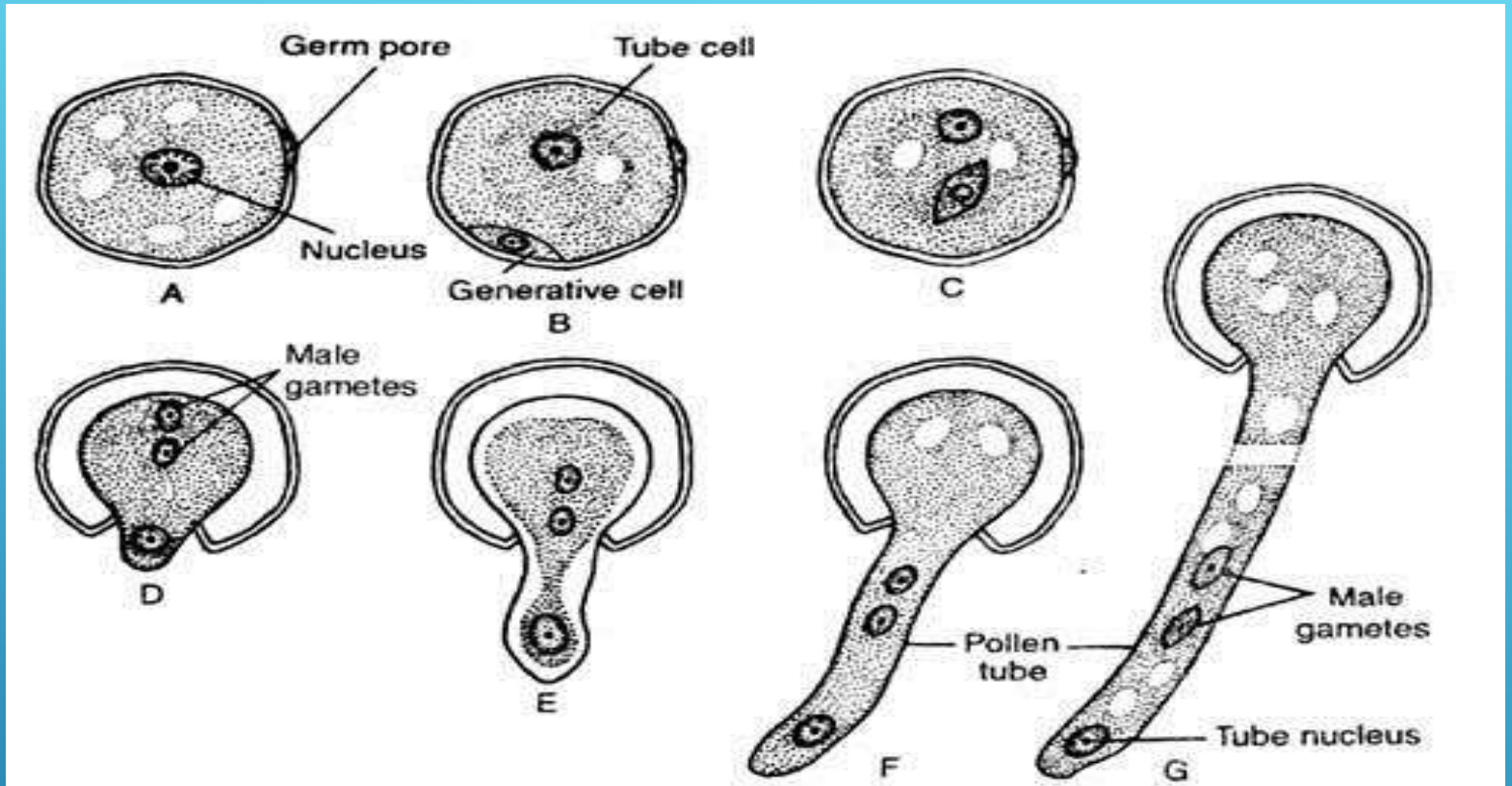


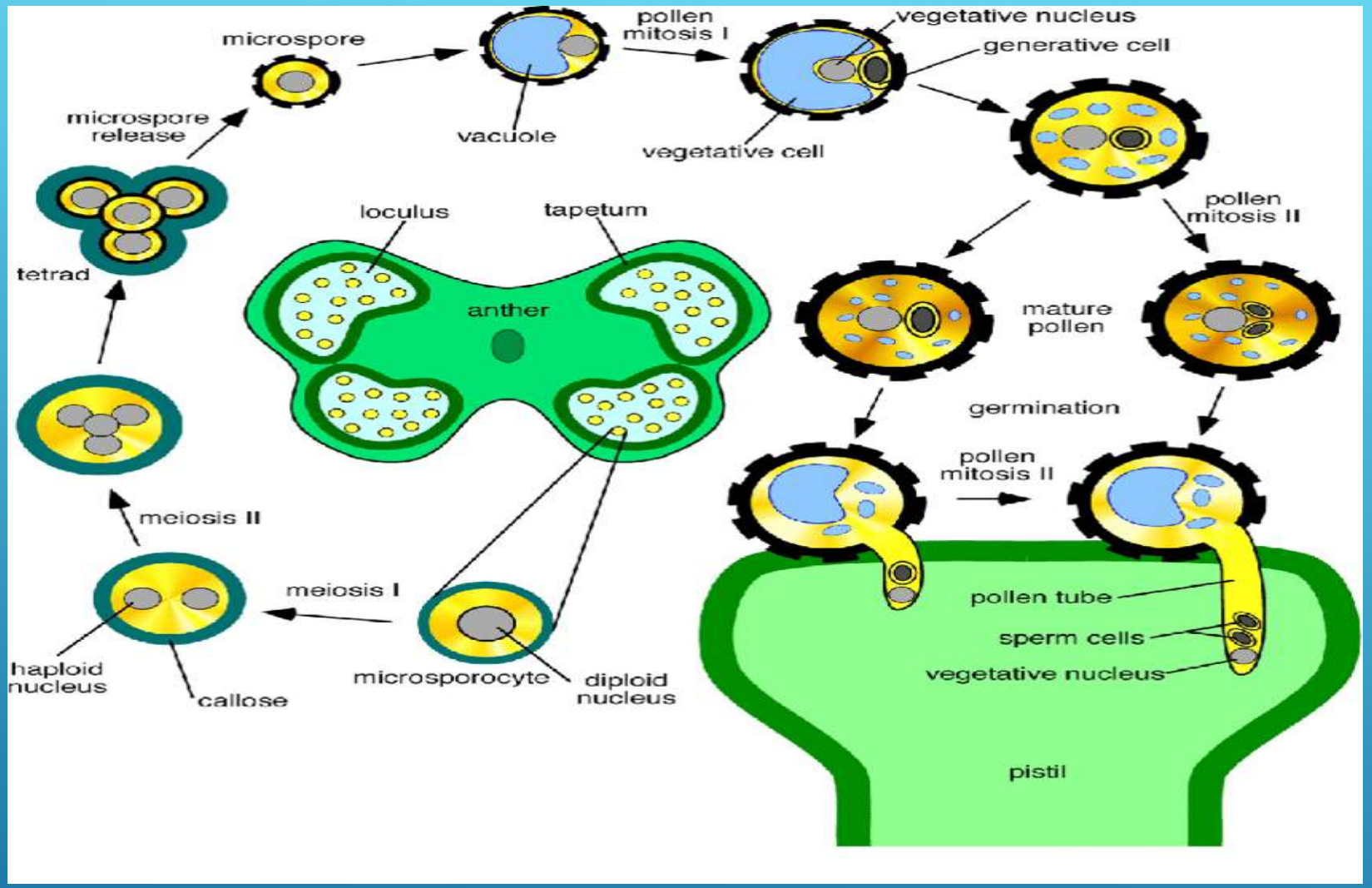
Fig. 3.5 : A-G. Germination of the pollen grain and development of the male gametes

Germination of Pollen grain and development of male gametophyte

https://www.biologydiscussion.com/wp-content/uploads/2017/04/clip_image010-23.jpg

- The second division i.e., the division of generative cell, may take place either in the pollen grain or in the pollen tube which develops through germ pore after pollination.
- The nucleus of the vegetative cell is commonly known as tube nucleus. 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. Sooner or later it may be degenerated completely.
- Tube nucleus was thought to have significance in direction of pollen growth but according to recent workers, it is purely non-functional vestigial structure.

Development of male gametophyte



Schematic diagram illustrating pollen development

https://www.researchgate.net/profile/david_twell/publication/239761143/figure/fig3/as:667807582797826@1536229143386/schematic-diagram-illustrating-pollen-development.png