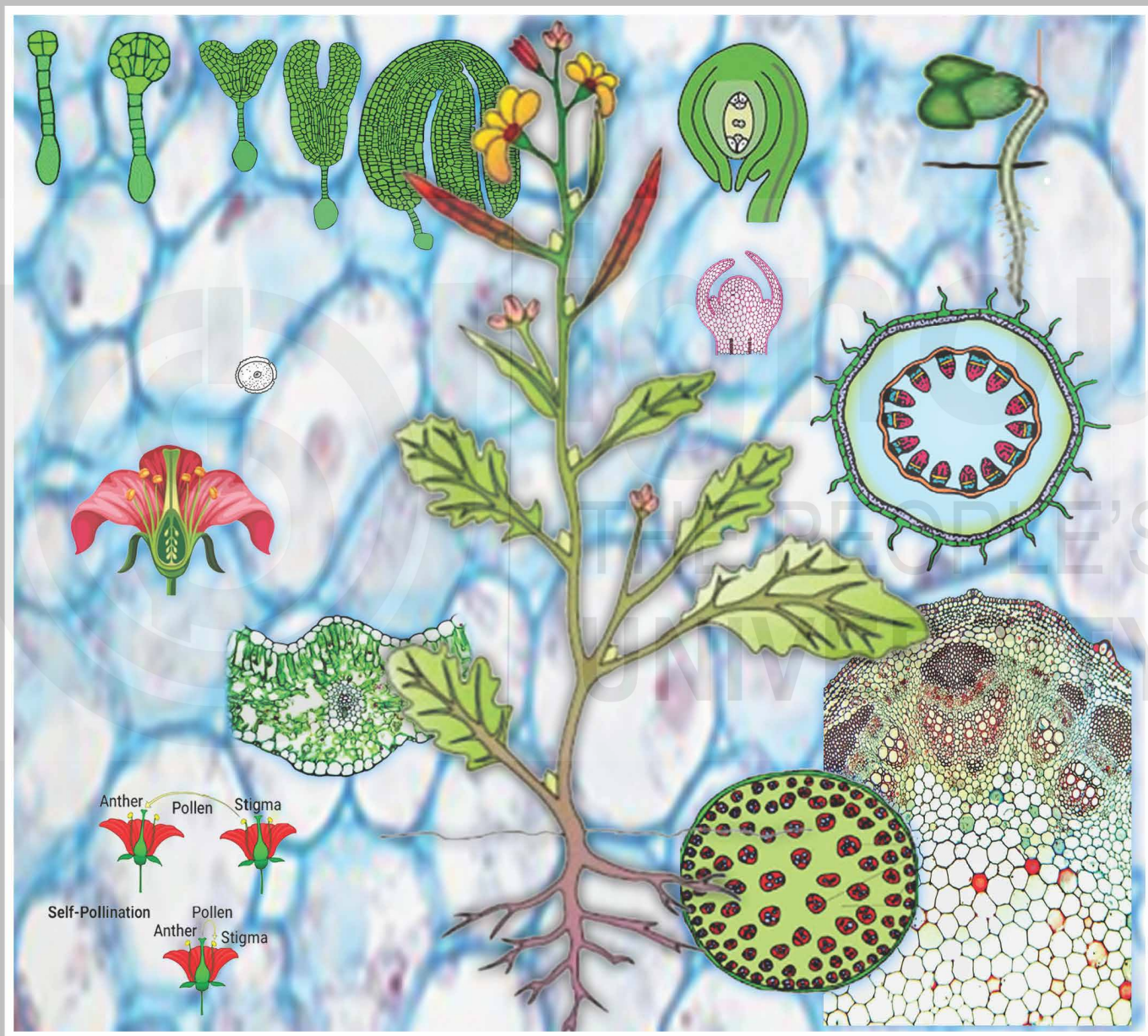




“शिक्षा मानव को बन्धनों से मुक्त करती है और आज के युग में तो यह लोकतंत्र की भावना का आधार भी है। जन्म तथा अन्य कारणों से उत्पन्न जाति एवं वर्तमान विषमताओं को दूर करते हुए मनुष्य को इन सबसे ऊपर उठाती है।”

— इन्दिरा गांधी



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“Education is a liberating force, and in our age it is also a democratising force, cutting across the barriers of caste and class, smoothing out inequalities imposed by birth and other circumstances.”

— Indira Gandhi

BBYCT-135 PLANT ANATOMY AND EMBRYOLOGY

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PLANT ANATOMY AND EMBRYOLOGY

Volume 2 deals with the embryology of flowering plants. This volume contains Block 3 and Block 4 which are completely dedicated to plant embryology. In flowering plants, like all sexually reproducing organisms, development commences from a fertilized egg or zygote. It develops into an embryo which lies protected in the seed within the fruit. When the conditions are right the embryo develops into a plantlet. In this volume we have discussed the details of development of reproductive structures in plants. The first unit deals with the flower, the anther and the ovule, with structural organization in relation to the functional aspects of male and female reproductive organs. These are the main seats for the production of pollen grains and embryo sac.

After gametogenesis, male and female gametes are formed, followed by the crucial steps of sexual reproduction i.e., pollination and double fertilization, resulting in formation of zygote and the primary endosperm nucleus. Endosperm is the nutrient store house of embryo locked up in the seed. The morphological structure, cellular details and function of endosperm are given in detail.

Next unit familiarizes you with various events during the development of embryo. You will know the difference between monocot and dicot embryo.

In certain plants, embryos develop without sexual reproduction or fertilization. In these plants, the embryos develop from unfertilized eggs, or from other cells of the embryo sac or ovule. This means that embryos are not produced as the result of syngamy but via the process of apogamy or apomixis. Sometimes there is the production of more than one embryo in a seed which is called polyembryony. These extra embryos may be produced as a result of multiple syngamy (result of fertilization) or by the process of apogamy or apomixis. The event is commonly noted in angiosperms, for examples onion, groundnut, mango, lemon and orange.

The post-fertilization development of the ovule and the ovary results in the formation of seed and fruit respectively. You will also study about the development of seed and fruit, various kinds of appendages present on the seed and common seed dispersal mechanisms.

Objectives

After study of this volume you would be able to:

- ❖ illustrate the structure of flower and explain the process of microsporogenesis and megasporogenesis;
- ❖ explain and describe syngamy and triple fusion;
- ❖ explain and depict various agents of pollination and describe the sequence of events in the process of fertilization;
- ❖ describe the development of embryo through various stages (globular, torpedo, heart shape);
- ❖ specify the role of endosperm and its importance in embryo development;
- ❖ describe the phenomena of apomixes and polyembryony and discuss their significance and applications; and
- ❖ summarize the various events involved in the development of seeds and fruits along with their dispersal mechanism.

BBYCT-135
PLANT ANATOMY AND
EMBRYOLOGY

Block

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BLOCK 3 : FLOWER TO FERTILIZATION

Flowers represent the major reproductive organs that possess androecium or gynoecium and form the basis of next progeny. The stamens and pistils present in a flower play a major role in fertilization leading to the formation of embryo that develops into a new plant. This block contains three units that provide detailed information about structure of a flower, pollination and fertilization. Information about various developmental events such as formation of gametes pollination and fertilization is also included in this Block.

In Unit 9 detailed information about the development, structure and function of male and female reproductive parts, organogenesis has been included. Besides this, male and female reproductive parts i.e., anther, pollen grains, male gametes, ovary, ovule, types of ovules and the process of microsporogenesis and megasporogenesis are given in detail.

In Unit 10 the structure of pollen and pollination has been dealt. The transfer of pollen takes place with the help of various agents such as wind, water or animals. In this unit you will become familiar with different types of pollination, some of the important adaptation exhibited by plants for successful pollination, about the various agents involved in pollination.

In Unit 11 you will study various developmental events during pollen tube growth, features of pistil, pollen pistil interaction, fertilization, double fertilization. Some factors in the stigmatic surface prevent the foreign or incompatible pollen from germinating and forming the pollen tube thus causing a rejection reaction or incompatibility and various methods to overcome incompatibility has been also described.

Objectives

After study of this block you should be able to:

- ❖ illustrate the structure of flower, ovary, ovule, types of ovules;
- ❖ explain the process of microsporogenesis and megasporogenesis;
- ❖ explain and describe syngamy and triple fusion;
- ❖ explain and depict various agents of pollination describe various events in the process of fertilization; and
- ❖ describe different types of incompatibility; identify various methods to overcome incompatibility.

UNIT 9

STRUCTURAL ORGANIZATION OF FLOWER

Structure

9.1	Introduction	9.6	Pollen Sterility
	Objectives		Nuclear Male Sterility
9.2	The Flower		Cytoplasmic Male Sterility
	Various Parts of Flower	9.7	Structure of Ovary
	Organogenesis and Development of Flower		Ovule
	Organogenesis during Flower Formation		Structure of Ovule
	ABC Model of Flower Organization		Types of Ovules
9.3	Structure of Anther	9.8	Megasporogenesis
	Tissue Organization in Anther	9.9	Structure and Organization of Mature Embryo (Polygonum Type)
	Development of Anther Wall Layers		Structure of Embryo Sac
	Syncytium Formation		Development of Embryo Sac
	Microsporogenesis		Haustorial Behavior of Embryo Sac
9.4	Structure of Pollen	9.10	Summary
	Development of Pollen Wall	9.11	Terminal Questions
9.5	Male Gametophyte	9.12	Answers
	Pollen Variants		
	Pollinia		

9.1 INTRODUCTION

In angiospermous plants, the vegetative phase of growth is followed by reproductive growth. The reproductive organs in plants are found enclosed in flowers. The vegetative shoot apex undergoes morphogenetic changes and produces a flower or inflorescence (group of flowers). This unit provides a detailed account of various parts of a flower and the processes involved in

floral development of flower. The detailed information regarding the development, structure and function of male and female reproductive parts, has been included in this unit. Besides this, details about female reproductive organs i.e., ovary, ovule, types of ovules and the process of megasporogenesis are provided in great detail this unit.

Objectives

After studying this unit you would be able to:

- ❖ describe different parts of the flower and anatomy of the floral organs;
- ❖ explain morphogenesis and organogenesis events occurring during development of flower;
- ❖ describe the structure of anther, male gametophyte and microsporogenesis;
- ❖ describe the structure of ovary, ovule and types of ovule;
- ❖ explain the process of megasporogenesis; and
- ❖ describe the structure and organization of mature embryo sac and types of embryo.

9.2 THE FLOWER

Flower is the reproductive structure found in angiosperms. It is also referred to as a modified-shoot containing the reproductive organs. The leaves or sporophylls get modified and get organized around a central stalk. The vegetative tip undergoes changes and develops into a floral tip. The localization of the flower can be lateral in an axil of leaf or terminal on the shoot. A flower in solitary or more than one are arranged in group to form an inflorescence. The central axis of the flower is the thalamus or receptacle that bears both sterile and fertile appendages in whorls. The sterile appendages include sepals and petals. Sepals form the outer whorl called calyx and petals form the inner whorl called corolla. The condition in which the sepals and petals look alike both whorls are together called **perianth**, made up of **tepals**. The stamens and carpels are the fertile appendages. Stamens located inside the corolla bear anthers which produce pollen grains having male gametes. Carpels or pistil located centrally in the flower bears ovaries that enclose ovules. The stamens are collectively referred to as **androecium** while carpels are collectively referred as **gynoecium**. Flowers that contain both androecium and gynoecium are called **hermaphrodites** (androgynous). If both male and female flowers are borne on individual plant it is called **monoecious**. Plants with male and female flowers borne on separate individuals are termed **dioecious**. A flower having all the four whorls, namely calyx, corolla, androecium and gynoecium, is referred as **complete flower** while that lacking any one of the four whorls is called as an **incomplete flower**.

A flower having both stamens and carpels is referred as perfect or **bisexual flower**. The flower having only either stamens or only carpel is referred as imperfect or unisexual flower. The flower in which only stamens are present is

referred as **staminate flowers** while those in which only carpels are present are called as **carpellate or pistillate** flowers.

The flowers are present in wide diversity in angiosperms. In some plants, flowers bear bright coloured and fragrant petals (example - rose), while in others perianth is non-distinguishable, reduced or absent (example- mulberry). The size, symmetry, colour and odour depends upon the pollination mechanism (transfer of pollen grains from stamens to stigma by various agents such as air, water, wind, insects, birds, bees and bats etc.).

9.2.1 Various Parts of Flower

Now you would study about various parts of flower. The flower stalk is a pedicel, and the very end of the axis is receptacle where other parts are attached. There are four types of floral appendages sepals, petals stamens and carpels which makes a complete flower. We will study them in detail (Fig.9.1).

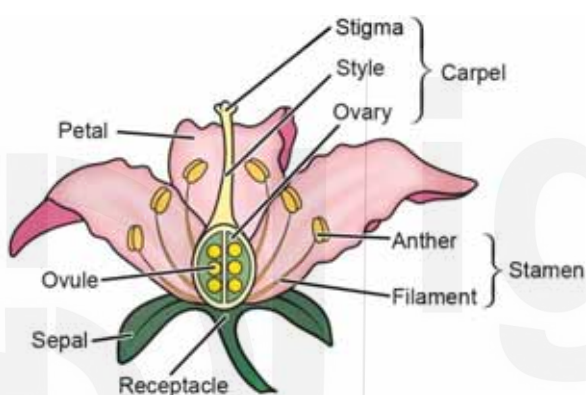


Fig. 9.1: Structure of a flower depicting different parts, such as androecium and gynoecium.

Calyx

Sepals that form the outermost whorl of flower, constitutes the **calyx**. They are modified leaves which enclose the other parts of flower and are mostly green in color. They may be brightly coloured in some species. In *Salvia splendens*, calyx is bright red in color. They are the thickest, toughest part of the flower and protect the bud as it develops into flower. They help in keeping the bacterial and fungal spores away, maintaining high humidity and preventing insect feeding. The petaloid condition of sepals is generally associated with loss of corolla as in silver oak. In members of Liliaceae, both sepals and petals look similar and are ornamental. In *Euphorbia* both sepals and petals are absent-the naked male and female flowers are grouped to form an inflorescence called cyathium which is surrounded by ornamental bracts. Sepals are mostly free, i.e. polysepalous, but in some flowers those are fused to form gamosepalous calyx.

Corolla

Petals present inside the calyx form the corolla. They are generally large, bright coloured and showy. However, they get generally reduced in water and wind pollinated flowers. Petals exhibit diversity in shape, size, colour and arrangement. They may be present free or fused to form a tube like structure. In orchids, petals are coloured and have symmetry that mimics moths and butterflies that pollinate them. In some plants petals are leaf-like, broad, thin

and flat. They attract insects which help in pollination. Petals in some flowers are fused to represent gamopetalous corolla.

Androecium

Stamens enclosed within the inner whorl of corolla form the androecium. The stamens may present in free form or in whorls of one or two or many. The stamens may be fused among themselves or with petals. When stamens are fused to form one group, the condition is called **monodelphous** (*Hibiscus*). Here the stamens fuse laterally to form a staminal tube. When stamens are fused to form two groups, the condition is called **diadelphous** (*Pisum*). When stamens are fused to form several groups, the condition is called polyadelphous (members of family Rutaceae). In some flowers stamens may be reduced or sterile, called as **staminodes**.

Each stamen bears a filament that bears anther. The anther generally is a four lobed structure having pollen grains. Each pollen grain produces two male gametes or sperms.

Gynoecium

The female reproductive part of the flower, made up of one or more carpels, is referred as gynoecium. If more, the carpels are free or fused. The gynoecium or pistil is generally distinguishable in three parts, namely ovary, style and stigma (Figure 9.2). Ovary encloses one or more ovules each of which bears the female gametophyte. Ovary continues to form a tube-like style that ends in stigma. After pollination, pollen grains germinate on stigma resulting in the formation of pollen tubes that carry sperms to the female gametophyte.

The fusion of various floral appendages is called as **adnation**. In Asclepiadaceae and Orchidaceae, the stamens and carpels are united completely or partially to form gynostegium.

In the past, botanists believed that the angiosperms with catkin-like inflorescence are the most primitive flowering plants. This is because their inflorescence has superficial resemblance to gymnosperm cones. The reduced flowers in these are comparable to reproductive organs of gymnosperms like *Ephedra* and *Gnetum*. In contrast, evolutionary studies have indicated that wind pollinated flowering plants with catkin-like inflorescences are more advanced in comparison to insect pollinated flowers.

Morphologists consider flower as a determinate shoot, and its appendages (sepals, petals, stamens and carpels) as the modified leaves. The floral organs are homologous (similar in origin) to leaves but perform different functions in comparison to leaves. The appendages at nodes are closely placed where as internodes are suppressed and shortened. The evolution of advanced flowers involved compression of the receptacle, reduction in number of appendages, change in symmetry and loss of leaf like appearance.

SAQ 1

- Name the different whorls present in a flower and describe them.
 - Differentiate between staminate and pistillate flowers?
-

9.2.2 Organogenesis and Development of Flower

In the earlier units you have studied about various theories related to shoot apex organization. Earlier theories proposed that shoot apex in angiosperms have surface initial cells called apical cells. The cell zone below it is called central mother cells which give rise to the pith, and lateral derivatives form the peripheral zone (cortex). The centrally located cells of the meristem maintain themselves and actively contribute cells to other region of the meristem. The central cells of the apical meristem are considered as resting meristem. This meristem does not have any significant role during vegetative phase of development. This meristem becomes active during the formation of flower or inflorescence. According to Buvat (1952), the meristem has a cytological zonation in which the main histogenic role is played by lateral or subterminal zone, the ***anneau initial*** or initial ring. The central cell of the apex, the ***meristeme d'attente*** or resting meristem is supposed to play no histogenic role during vegetative phase of development. Belgian morphologist Gregorie (1938) believed that the floral meristem is a new structure which is not derived from vegetative meristem but from lateral buds. According to tunica corpus organization, the meristematic mantle gives rise to floral parts and contributes cells to parenchymatous core inside. Philipson (1947) claimed that the tunica-corpus organization exists in the reproductive apex. The vegetative stem apex gets transformed to form reproductive apex.

Irrespective of whether the vegetative shoot meristem has a tunica-corpus organization or cyto-histological zonation, the reproductive apex develops certain changes and becomes modified during flowering induction. The apex becomes more elongated and conical. The frequency of mitotic divisions increases. DNA synthesis also increases. The cells of the central core of the apex become highly vacuolated. The smaller cells form the outer covering or mantle. The floral apex develops a mantle-core organization. Large faintly stained cells of the vegetative apex become active and undergo rapid mitotic divisions. The region now possesses small densely stained cells and becomes part of the meristematic mantle which surrounds the central parenchymatous core. Plantefol (1947) gave the opinion that reproductive apex originates from transformation of vegetative apex. The *anneau initial* gives rise to foliage leaves in the vegetative phase, and produces sepals and petals in the reproductive phase. Stamens and carpels are derived from residues of the apex. Bersillon (1955) suggested that sepals are derived from *anneau initial* whereas floral appendages are derived from the meristematic mantle obtained from the *meristeme d'attente*.

In *Cleome* that bears inflorescence without a terminal flower, the *anneau* gives rise to lateral flower buds and bracts. The species in which inflorescence has a terminal flower (species such as of *Beta*), *anneau initial* remains inactive in the reproductive phase. The *meristeme d'attente* is responsible for generation of bracts and flowers. In some species, an intermediate situation has been noted. In these species lateral flowers are formed by *anneau* initial before the *meristeme d'attente* becomes active and forms the terminal flower.

Many authors disagree with the opinion that histological zonation of vegetative apex helps in the development of different parts of the flower. They suggest

that whole apex gets transformed to enter new phase of development. According to Philipson (1947) the basic function of the vegetative apex is promotion of longitudinal extension of the axis, while the reproductive apex produces different parts of the flower. The cells present between the zone of central mother cells and rib meristem zone show high mitotic activity. Later on central mother cells also undergo divisions and become small and rich in protoplast. All the cells above the rib meristem get added to tunica region and constitute a mantle. The mitotic activity and growth stops in the rib meristem. The reproductive apex bears pith made up of parenchymatous cells and core surrounded by mantle of meristematic cells. The floral parts i.e., bracts, axillary branches and flowers in inflorescence develop from meristematic cells. The mitotic division is followed by initiation in floral buds. A number of cytological changes such as increase in number of ribosomes, dictyosomes and endoplasmic reticulum have been noted.

9.2.3 Organogenesis during Flower Formation

The induction of flowering phase is accompanied by formation of leaf-like bracts and florets by the apex of the inflorescence. Various floral organs are formed in an acropetal manner with youngest organ near the apex. Sepals, petals, stamens and carpels originate by periclinal divisions in the layers of tunica. The surface layers undergo anticlinal divisions to keep pace with the growth in the tunica layers. In *Catharanthus roseus*, the reproductive apex gives rise to sepals first. The apex then enlarges and forms petals in whorls. The base of petals fuses to form corolla tube. Five staminal primordia are formed which then fuse with the basal region of the corolla. The five stamens appear as outgrowths of petals. Thereafter the staminal primordia get reduced. Subsequently, the diameter of the floral apex increases and cells of the periphery multiply to form the raised ring. Two carpel primordia are formed on the opposite sides of the ring. Primordia of sepals, petals, stamens and carpels are formed by periclinal divisions in the second tunica and outer corpus layers. The growth of each organ is initiated from the primordia (Figure 9.2). Apical growth is followed by growth of subapical meristems. The location (intercalary, marginal, adaxial), time of initiation and extent of meristem activity determine the diversity of floral organs in each species. In vegetative shoot, the apical meristem continues to develop into leaf primordia. In a developing flower, the apex is reduced following initiation of floral organs till it is lost or becomes inactive.

The lateral organs of the flower exhibit apical, marginal, plate and adaxial meristems. Sepals and petals grow lengthwise by apical and intercalary meristems and expand on all sides by the activity of marginal meristem. The organs become thick due to plate meristem. The adaxial meristem is present in sepals but absent in petals. Stamens show apical growth initially followed by intercalary activity (Fig. 9.2). The carpels show adaxial meristematic activity. This contributes to the thickness of the carpels.

In flowers having free carpels i.e. apocarpous condition, each carpel primordium appears as rounded buttress which resembles primordia of other floral organs or leaves. The primordium becomes peltate and a depression is formed at the tip. Unequal development results in the formation of abaxial lip

from which dorsal side of carpel is formed. The adaxial lip undergoes development to form 'sill' which later on forms margin of the carpel. In flowers with fused carpels, i.e., syncarpous condition, ovary is formed either through fusion and enlargement of carpel primordia or by joining of carpels and fusion of ovary wall to form a ring.

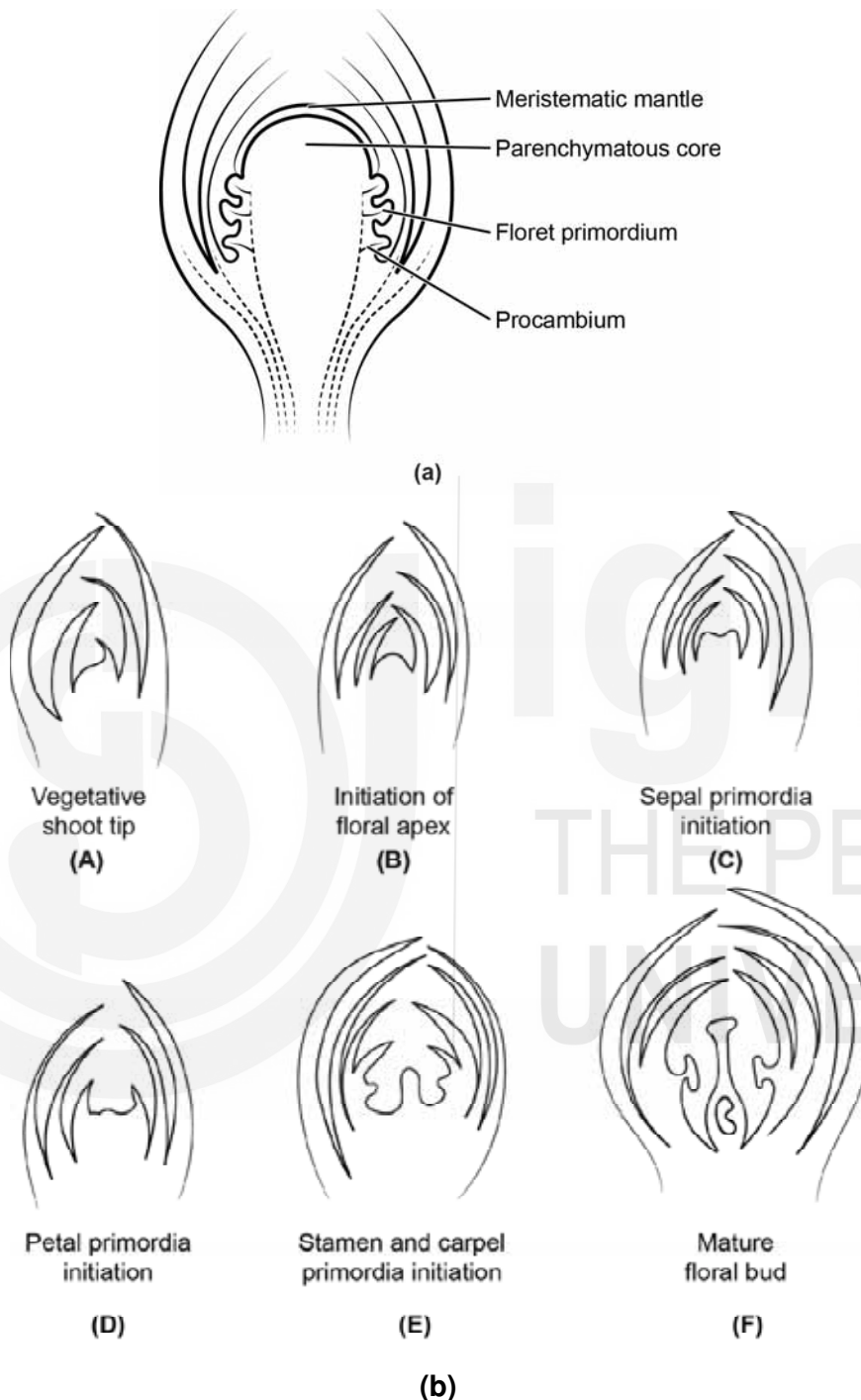


Fig. 9.2: a) L.S of floral apex; b) A-F; Development of flower from shoot axis.

9.2.4 ABC Model of Flower Organization

All the flowers show same basic developmental organization. They possess four types of appendages namely sepals, petals, stamens and carpels. In a typical eudicot flower, five of each appendage such as sepals, petals, stamens and carpels are found, while in monocots, typically three of each appendages is found. Some flowers also have dozens of petals, 90 or more stamens and numerous carpels.

The basic organogenesis of flowers is explained by ABC model. ABC model of flower development demonstrates the presence of three classes of genes that regulate the development of floral organs in angiosperms. Interaction between these genes induces the development of floral organs. It has been suggested that floral organs arise in four concentric rings or whorls and development of each whorl is a consequence of the expression of the specific temporal or spatial pattern of **homeotic genes**. These genes encode proteins that determine identity of the organ. In an angiospermous plant, four concentric whorls of organs are present in a flower. These are sepals (whorl 1), petals (whorl 2), stamens (whorl 3) and carpels (whorl 4). Each **homeotic** gene that induces the formation of floral organs has an effect on two groups of floral organs, i.e. sepals and petals, petals and stamens or stamen and carpels (i.e. genes A, B and C). In each whorl of a flower there is one or more homeotic genes and their cooperative functions determine the organ to be formed in that whorl. For example, the activity of class A genes is restricted to whorls 1 and 2. The class B genes have function in whorls 2 and 3. The class C gene functions in whorls 3 and 4.

Three genes namely A, B and C control the basic aspects of flower organization (Fig. 9.3). Genes A affect the outermost, lowest regions of the flower shoot apical meristem, genes C control the uppermost, central region and genes B overlap the other two. Class A genes operate in whorls 1 and 2, class B operates in whorls 2 and 3, and class C operate in whorls 3 and 4. In the region where genes A are expressed, the primordia develop into sepals. The primordia in the upward and inward region are influenced by genes A and B and they develop into petals. The combination of genes B and C produces stamens, and in the center of the flower where genes C are expressed the primordia develop into carpels. This means that in the whorl 1 expression of class A genes induces the development of sepals. Interaction between class A and class B genes induces the development of petals in the whorl 2 (Fig. 9.3). Stamens are formed in the whorl 3 as a result of interaction between class B and class C genes. In the whorl 4, class C genes induce the formation of carpel. The ABC model was formulated by Coen *et al.* in 1991.

In *Arabidopsis* there are two genes in class A, two genes in class B and one gene in class C. The function of class A genes is confined to whorls 1 and 2. Similarly the function of class C gene is restricted in whorls 3 and 4. In the whorls 1 and 2 the function of class A genes prevents class C gene from functioning in the same whorl. Similarly the function of class C gene prevents class A genes from functioning in the whorls 3 and 4.

Honma and Goto (2001) stated that besides these three sets of genes some other genes are required for flower formation. They identified the fourth class of genes (gene E). The protein products of this gene interact with A, B, C genes. They proposed a model to show how these proteins bind to different pair of genes. Since the four pair of genes was involved in the formation of specific floral organs the model was termed as **quartet model**. About 100 MADS box genes exist in genome of *Arabidopsis* and these are known to play a role in differentiation of plant organs in addition to development of flowers.

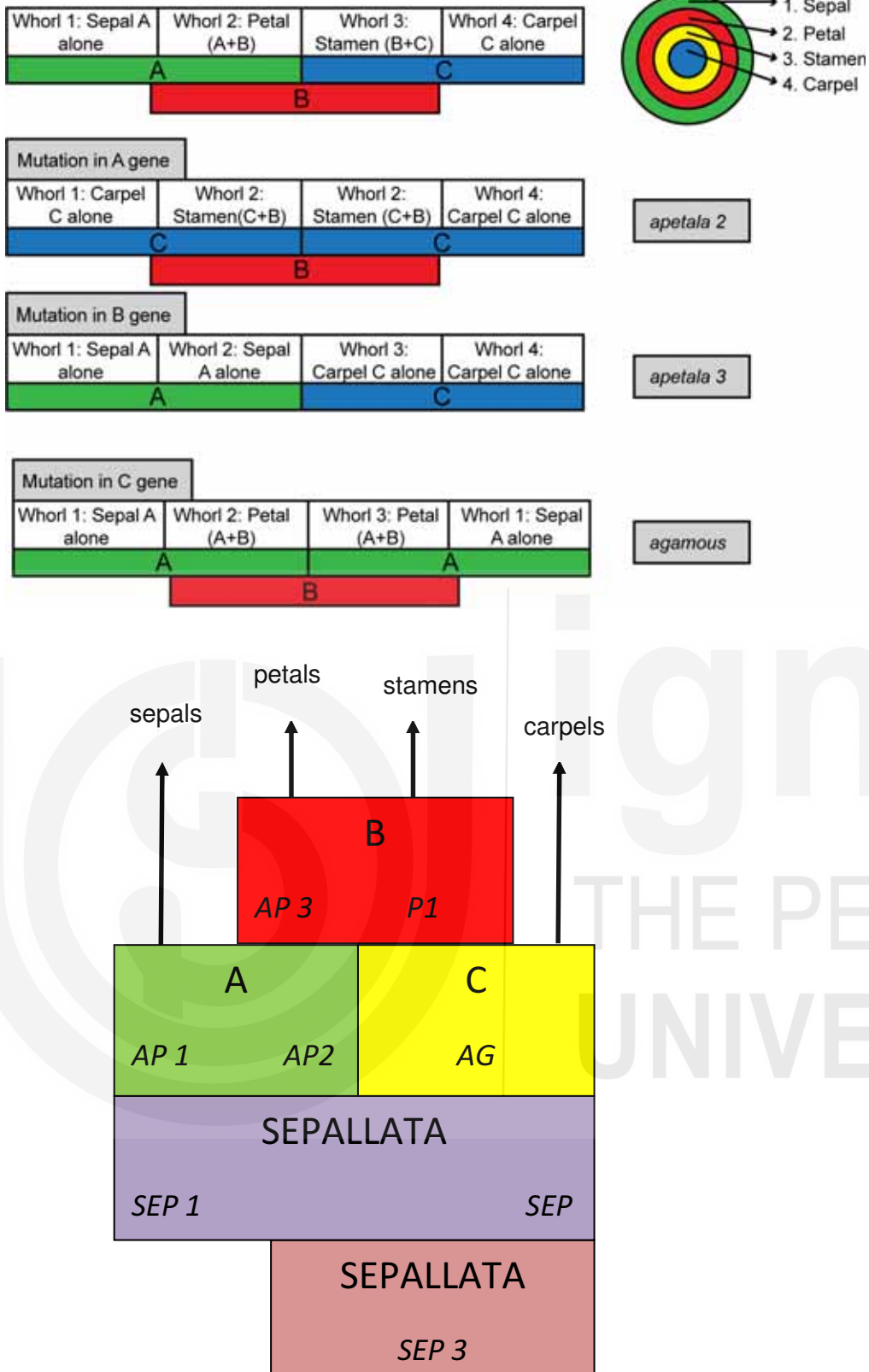


Fig. 9.3: ABC model of flower development (b) taken from Jack (2001).

This model was supported by studies in many types of aberrant flowers. For example if gene B is mutated and non-functional, sepals are produced in the outer part of the flower and carpels are formed next to it but inside the flower. The flower does not produce petals or stamens. There is outer whorl of sepals followed by inner whorl of sepals instead of petals. Similarly there is a whorl of carpels instead of stamens followed by inner whorl of carpels. The flower hence has all four whorls, but only sepals and carpels in the wrong sites. A mutation that causes an organ to develop in an unexpected site is called

homeotic mutation. Mutation in gene A results in carpels in whorl 1 (where sepals are expected) and stamens in whorl 2 (where petals are expected). Study of mutants helps in understanding the function of various genes.

SAQ 2

- Describe the process of transformation of a shoot apex during development of flower.
- Three genes regulate the stages of flower development. Explain.

9.3 STRUCTURE OF ANTHER

The structure in which pollen is produced is called anther. A mature anther is a four lobed structure having four pollen sacs or microsporangia (tetrasporangiate). An anther is composed of diploid cells. In each pollen sac mass of enlarged cells called microspore mother cells or microsporocytes are present. These cells enlarge and undergo meiosis to produce microspores.

9.3.1 Tissue Organization in Anther

Anther is a four lobed structure (Fig.9.4). A young anther consists of homogenous mass of meristematic cells surrounded by epidermis. A group of two or more hypodermal **archaesporeial** cells differentiate in each lobe (Fig. 9.4).

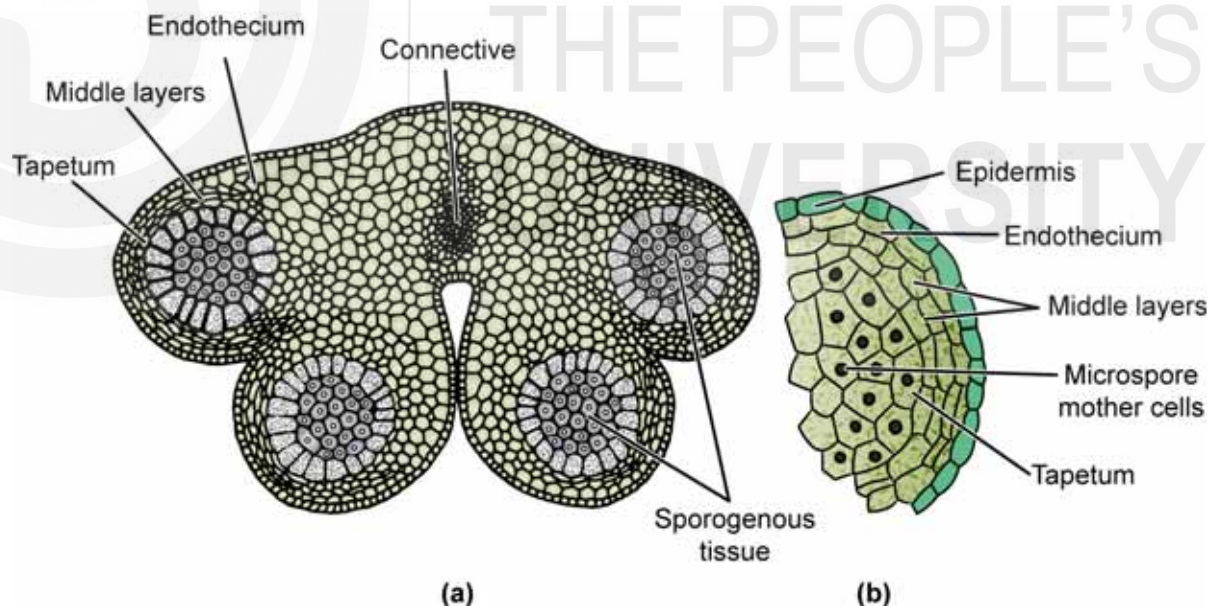


Fig. 9.4: a) T.S of mature anther showing tetrasporangiate structure; b) Layers of anther wall (enlarged view).

These cells possess dense cytoplasm and have prominent nuclei. The archesporial cells undergo periclinal division (divide in a plane parallel to the outer wall of the anther lobe). Such division leads to formation of outer primary parietal cell (PPC) towards the epidermis and inner primary sporogenous cell (PSC) towards inside. The PPCs undergo repeated periclinal and anticlinal divisions (division at right angle to the surface) to produce 2- 5 layers of the

anther wall. The primary sporogenous cells undergo mitotic divisions to produce many sporogenous cells that round off to form microspore mother cells (MMC). The microspore mother cells or microsporocytes enlarge develop thick callosic wall and undergo meiosis to produce four microspores. The microspores initially remain attached to form a tetrad but later on separate and develop into mature pollen grain. The process of development of microspores from microspore mother cells is referred to as **microsporogenesis**.

9.3.2 Development of Anther Wall Layers

The anther primordium is parenchymatous. It becomes 4-lobed and in each lobe archesporial cells are differentiated below the epidermis (Fig. 9.5).

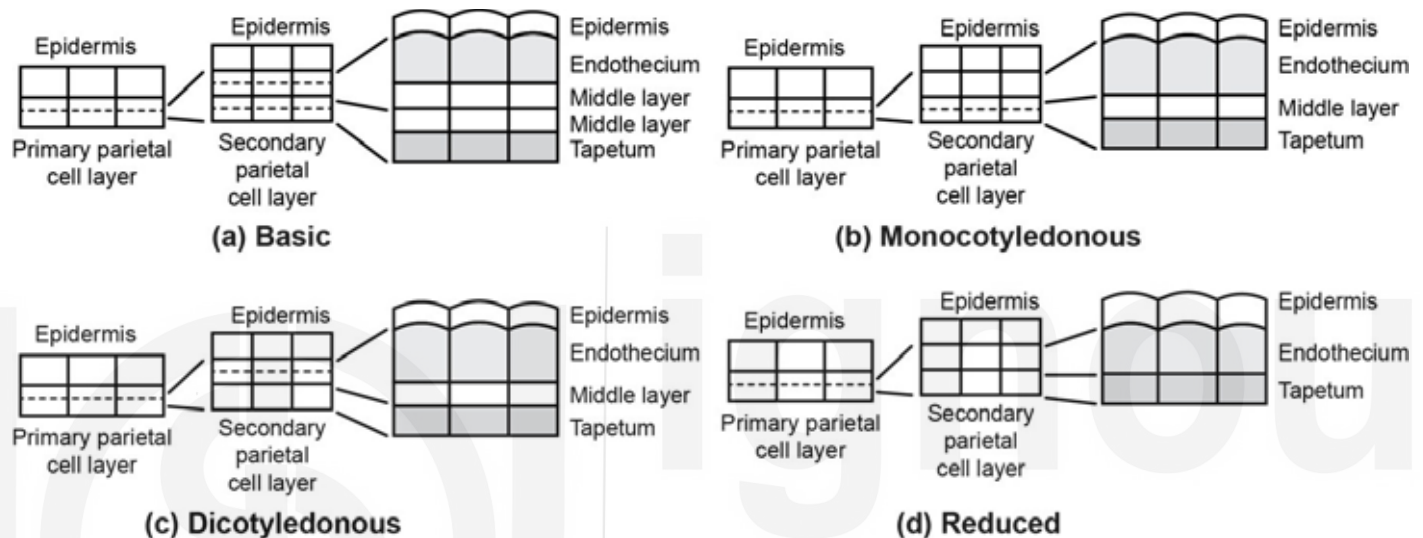


Fig.9.5: Development of anther wall layers.

The hypodermal archesporial cells divide periclinally to form outer primary parietal cells and inner primary sporogenous cells. The primary parietal cells divide periclinally and anticlinally to form anther wall layers. At the same time, the primary sporogenous cell divides repeatedly in all planes to form sporogenous cells.

A mature anther wall in most of the angiosperms is normally made up of five or more layers. The outermost layer is epidermis which is followed by endothecium, middle layers and tapetum. The cells of the **epidermis** undergo anticlinal divisions, become stretched and flattened. These cells provide protective covering to the internal tissues. A layer of cells lying next to epidermis forms the **endothecium**. The endothecium differentiates in each of the four anther lobes and helps in the dehiscence of the anther. The endothelial cells are radially elongated. The cells get enlarged and develop cellulosic thickenings. The wall thickenings are recognized as fibrous bands and provide tensile force that pulls back the anther wall from the line or region of dehiscence. During dehiscence the outer and inner tangential walls of the endothelial cells play an important role. The inner tangential wall appears thick while the outer wall appears thin. The inner wall gets thick due to the presence of cellulose and absorbs more moisture which results in the rupturing of the anther wall. The rupturing occurs at the weak part of the anther wall. Hence the hygroscopic expansion of the outer and inner tangential wall of the endothelial cells help in dehiscence of anther to liberate pollen.

Inner to endothecium lie 1-3 of cells form the **middle layers**. These layers originate from primary parietal cell (PPC). The cells of the middle layers are generally rich in food reserves such as starch. The cells are ephemeral and get compressed between endothecium and tapetum in a mature anther

Tapetum forms the innermost layer of the anther wall that surrounds the sporogenous tissue (See Fig. 9.4). It is usually composed of single layer of metabolically active cells. It acts as the nutritive tissue that helps in the development and maturation of microspores. Tapetum towards the outer side is derived from layer of primary parietal cells, whereas on the inner side it develops from the cells of the connective tissue. It thus has a dual origin. In young anther, the tapetal cells remain in contact with the sporogenous tissue through plasmodesmata. The connections get dissolved after-microspore mother cells (MMCs) enter meiosis. Tapetum is involved in nourishment of developing MMC and microspores inside. It also plays a role in the formation of exine, tryphine and pollenkitt. It also secretes sporopollenin which forms an important constituent of the wall of pollen grains.

During gametogenesis, the cells of the tapetum increase their DNA content due to irregular mitotic divisions. They become two, four, eight or sixteen nucleate and their nuclei undergo fusion to form polyploid nuclei. The tapetal cells become polyploid either due to endomitosis, polyteny or by the formation of restitution nuclei. In some angiosperms, the nuclear divisions are followed by periclinal divisions resulting in formation of multilayered tapetum. The development of tapetum is important for the development of pollen grains. If the tapetum fails to develop or function, the viable pollen grains are not formed. Spontaneous or artificially induced abortion of tapetum leads to pollen sterility.

Tapetum is of two types- glandular and amoeboid. In glandular or secretory tapetum, the cells maintain their individuality and position but breakup at later stages of microspore development. In amoeboid tapetum the cells change their shape and position during ontogeny to form a **periplasmodium**.

In **amoeboid tapetum** (invasive or periplasmopodial) inner tangential and radial walls break, protoplasts enlarge and their fusion results in the plasmodium spreading in the anther cavity around the microspore mother cells (Fig. 9.6). The tapetal wall breaks due to release of hydrolytic enzymes by dictyosomes. The amoeboid tapetum is found more often in monocotyledons. At the microspores tetrad stage, the tapetal cytoplasm produces the enzyme callase which breaks down the callose wall around the microspore tetrads, releasing the microspores into the periplasmodium. The periplasmodium degenerates, dries and the pollen grains become coated with pollenkitt material towards the end of the pollen development.

The **secretory or glandular** (parietal or cellular) type of tapetum remains intact throughout the microspore development and provides nutrition to the developing microspores. The tapetal cells secrete and release substances to the anther sac from the inner walls. It is commonly found in dicotyledons. RNA and protein synthesis takes place in the tapetal cells during premeiotic and meiotic period. The cells of parietal tapetum show high metabolic activity and possess well developed mitochondria, ER and plastids. The tapetal cells

develop pro-orbiscles. Sporopollenin is deposited on the pro-orbiscles. The orbiscles (also called Ubisch bodies) play a role in the development of exine of pollen. Hence tapetum is the most active layer of the anther wall. It synthesizes certain substances such as fibro-granular proteins, carotenoid containing lipid droplets which get released in the anther locule during maturation of pollen grains. The fibro-granular proteins enter the exine and get stored in the region of bacula. The carotenoid containing lipid granules spread over exine and help in pollination. These are also referred to as pollenkit.

The primary sporogenous cells (PSC) are formed after the periclinal divisions of the archaespore cells. The PSCs undergo mitotic divisions and increase in number to form many sporogenous cells before functioning as microspore mother cells (MMCs) or can directly function as MMCs.

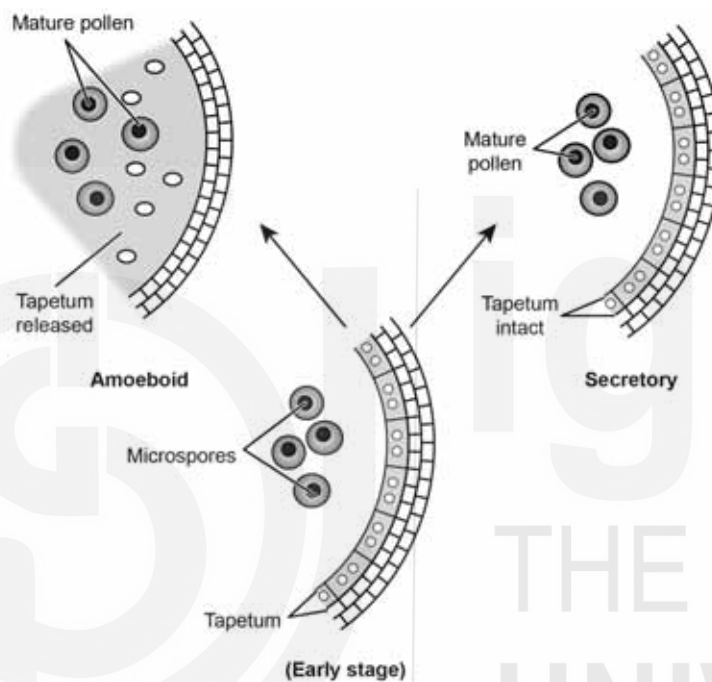


Fig. 9.6: Types of tapetum.

9.3.3 Syncytium Formation

In young anther, the different cell layers i.e. anther wall layers, tapetum and sporogenous tissue exhibit plasmodesmatal connections between the adjacent layers. The flow of nutrients between different layers takes place through these connections. After the onset of meiosis, these connections get affected and only the cells of the same layer continue to have plasmodesmatal connections. The callose deposition begins to occur inner to the cellulosic cell wall in the sporogenous tissue. The plasmodesmatal connections between the sporogenous cells become wider to form cytoplasmic bridges. Each MMC becomes enclosed in a callose wall. The callose deposition occurs at the corners of the cells. The cytoplasmic connections become wide to allow the free passage of cytoplasm and organelles between meiocytes. Hence hundreds of MMCs in each microsporangium constitute to form a single functional entity called syncytium.

9.3.4 Microsporogenesis

With the progress of meiosis, callose wall extends to regions occupied by cytoplasmic channels. After completion of meiosis, the callose wall extends to

the separating wall of the microspore tetrads. Thus, individual microspore gets separated. The callose wall acts as a selective barrier for passage of larger molecules such as proteins and peptides. The arrangement of microspores in a tetrad varies. The arrangement can be linear, isobilateral, tetrahedral, decussate or T-shaped (Fig. 9.7). The variation occurs in the formation of cell wall (cytokinesis) separating the microspore. In successive types of cytokinesis, the two nuclei formed after the first nuclear divisions get separated by a wall to form dyad which further undergoes division followed by wall formation to form an isobilateral, decussate or T-shaped tetrad. Simultaneous type, which is more common, the first nuclear division is not followed by wall formation. The walls are laid only after the formation of four nuclei as in tetrahedral tetrad.

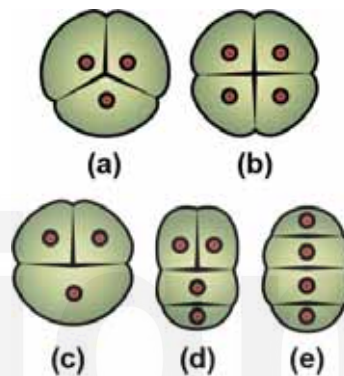


Fig.9.7: Arrangement of microspore in tetrad (a-e).

Meiosis is associated with the reorganization of the cytoplasm in MMCs and microspores (Fig. 9.8).

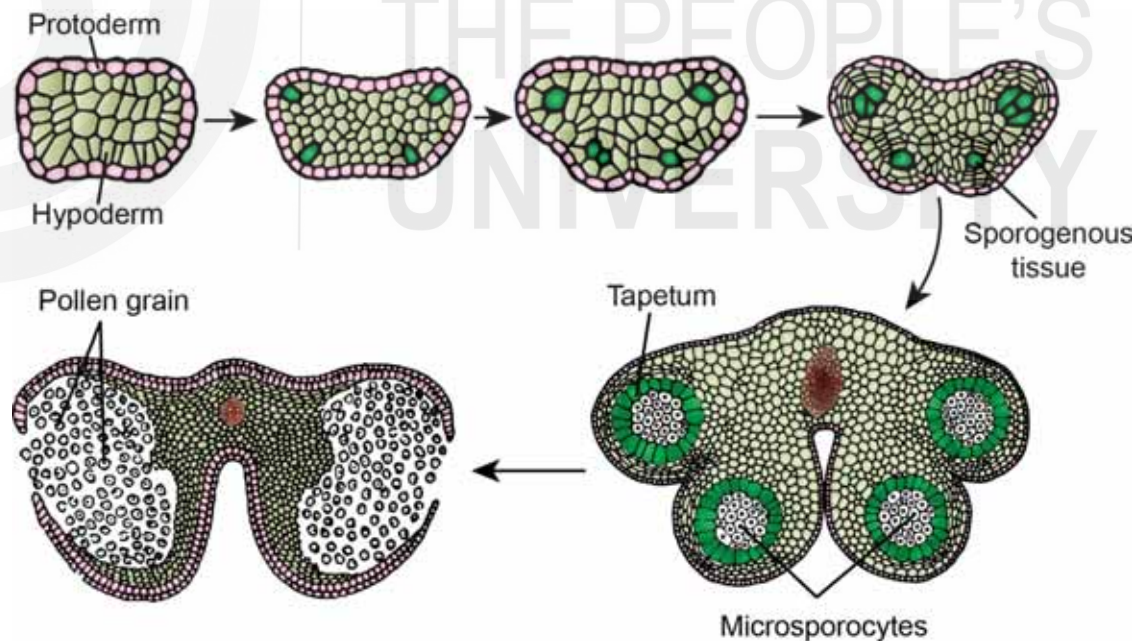


Fig. 9.8: Stages in microsporogenesis.

MMCs show high metabolic activity. The rate of synthesis of RNA and proteins gets reduced after meiosis. The cells undergo dedifferentiation. The ribosome count declines and the mitochondria and plastids undergo dedifferentiation. They lose internal membrane system and become spherical bag-like structures. The small pockets of cytoplasm get enclosed by membranes. The microspores resume synthetic activity after meiosis. The synthesis of proteins

and RNA get initiated, the ribosome number gets restored and mitochondria and plastids redifferentiate to their normal structure with internal membrane system.

SAQ 3

Briefly enlist the various layers of anther with their function.

9.4 STRUCTURE OF POLLEN

The pollen wall is made up of two layers - the inner pectocellulosic layer called **intine** which gets degraded during acetolysis and the outer pectic polysaccharide layer called **exine** which is resistant to acetolysis and physical and chemical degradation (Fig. 9.9 a). Exine is composed of sporopollenin which is a highly resistant material derived from carotenoids after polymerization. Exine is differentiated into two layers- outer sculptured layer, the sexine and the inner non-sculptured layer- nexine. Intine is also differentiated into two layers. The outer pectic polysaccharide layer called exintine and the inner cellulosic layer called endintine respectively.

9.4.1 Development of Pollen Wall

After completion of meiosis, the primeexine is formed. The microspores are enclosed in wall made up of callose. The formation of pollen wall occurs in two phases. In the first phase, the wall material is contributed by the cytoplasm of the spore (gametophytic) during the tetrad stage. The second phase occurs during the release of microspores. In this phase, the wall materials are contributed by the tapetal cells (sporophytic) in addition to the spore cytoplasm. The callose is dissolved through enzymatic degradation and microspores are released. The wall materials are provided by tapetal cells. The primeexine is cellulosic and the cellulose microfibrils of this layer are deposited in between the convoluted plasmalemma of the microspore and the callose wall. The primeexine is discontinuous and the gaps form the germ pores. With the growth of the tetrads, the columns of the lamellae are deposited in these gaps at the surface of plasmalemma. The columns are called probaculae.

The spore cytoplasm synthesizes the precursors of sporopollenin which gets polymerized and deposited on the surface of lamellae. The columns are called baculae. The sculptured part of the exine is made up of radially oriented rod like baculae (columella) which remain open (plitate grain) or may be covered by a roof or tectum (tectate grain). The distribution and orientation of the bacular columns vary according to the pattern of the exine. The baculae may be enlarged above and stand free or fused together to give a wall arranged in a reticulate pattern. The lower ends of the baculae spread sideways in the cellulosic layer of the primeexine and coalesce to form the foot layer. The top of the baculae spreads sideways in all directions to form the tectum or enlarged knob-like structures (Fig. 9.9 b). In the tectate grain, the voids between baculae open to the outside through perforations termed as micropores. The exine is present on the entire surface of the pollen grain except the germ pores or germinal apertures.

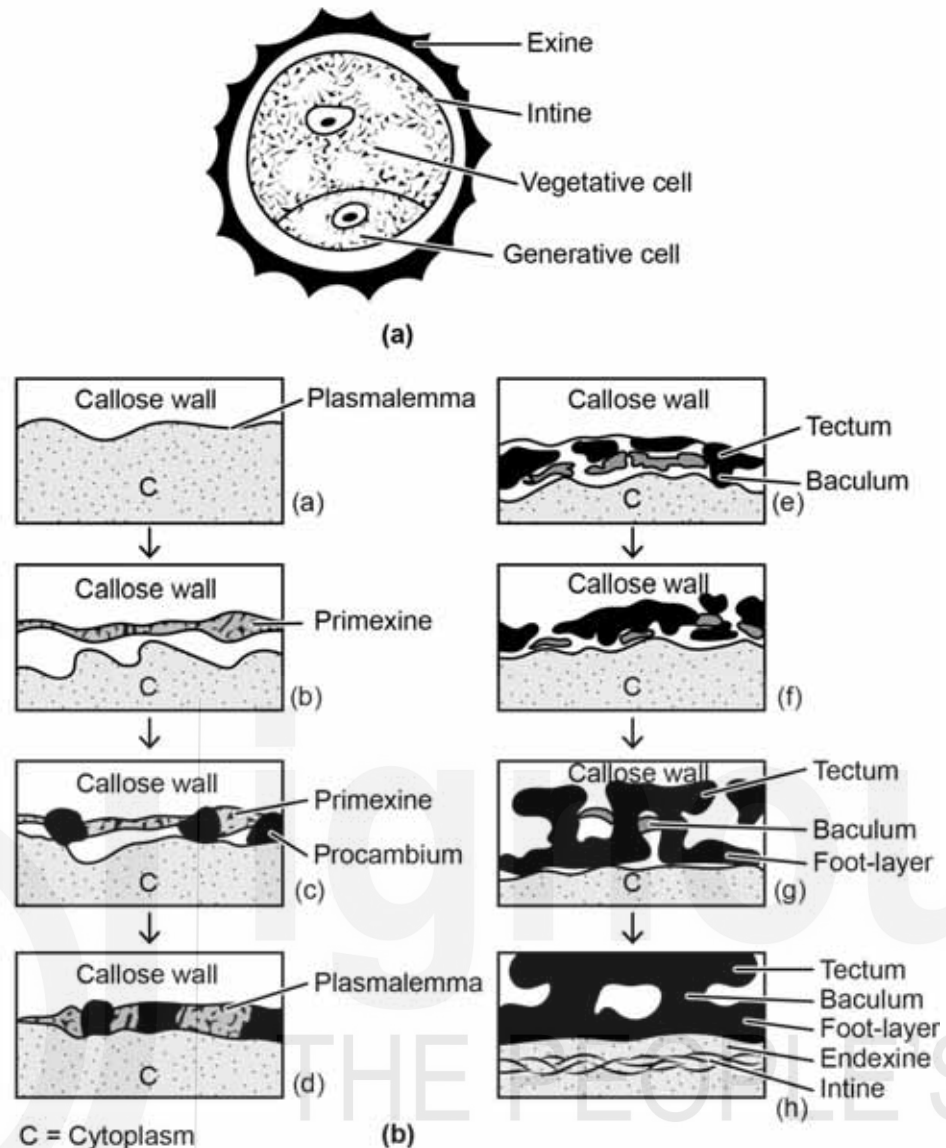


Fig. 9.9: a) Structure of pollen; b) Pollen wall development- a. callose surrounds the plasmalemma, b : primexine gets deposited between the callose wall and plasmalemma, c-d : probaculae appear in the primexine, e : formation of baculae and tectum, f-g : disappearance of callose wall and formation of exine, h : well developed wall with all the layers.

The pollen grains synthesize intine after they get liberated from tetrad after dissolution of the callose wall. The formation of intine occurs by the activity of the dictyosomes. During the early growth of intine, the thickening of the inner layer of the exine (endexine) continues and the lamellar materials and the sporopollenin precursors pass through the developing intine. Certain proteinaceous plates or ribbons present in the vicinity of the germ pores show enzymatic activity. Intine proteins are produced by pollen cytoplasm (gametophytic origin), while the exine proteins are contributed by tapetal cytoplasm (sporophytic origin). After the breakdown of tapetal cells, the enclosed proteins and lipids are released into the sporangial cavity as pro-*ubisch* bodies and eventually get deposited in the depressions of the exine. In tectate grains, the protein fraction passes through the microspores of the tectum and accumulates in the spaces between the baculae. Thus the intine and exine proteins are gametophytic and sporophytic in origin. The pollen wall proteins are involved in recognition-rejection reaction on stigma (germination of pollen only of same species). Incidentally, those pollen wall proteins are also responsible for allergenic reaction in some human individuals.

Pollen grains have been characterized on the basis of shape, size, and thickness, ornamentation of exine and the number and type of apertures. Two types of apertures have been known- porate and colpate. The latter has elongated furrows along the axis. In some plants the pollen grains are exceptionally different. Example- in aquatic plants, the pollen grains are filiform i.e. the microspores are linearly arranged in a tetrad and on separation they become elongated and filamentous.

The lipoidal layer found on the outside of the mature pollen in insect pollinated species is known as **pollenkitt**. The layer also consists of flavanoids, carotenoids, and degeneration products of the tapetal proteins. The carotenoids give a yellow color to the material. It is believed to act as insect attractant and plays a role in pollen dispersal. It also protects the pollen from effects of UV radiation.

9.5 MALE GAMETOPHYTE

Microspores develop into microgametophytes. In all the angiosperms, the male gametophyte or microgametophyte is small and consists of three cells located within the cell wall. The microspore nucleus migrates to a position next to the wall. It divides mitotically to produce a large **vegetative cell**, and a small lens-shaped **generative cell** which subsequently divides further to form two sperm cells or male gametes (Fig. 9.10). The position of the generative cell is related to the microspore nucleus and the orientation of the spindle. Unequal division of the microspore is related to spindle. The pole toward the pollen grain wall is flattened and is referred to as generative pole. Asymmetry of the spindle leads to formation of curved wall which separates the smaller generative cell from the larger vegetative cell following division the microspore is referred to as pollen grain. The generative cell soon gets detached near the wall and lies within the pollen grain cytoplasm. It assumes oval shape and gets surrounded by plasma membrane. The composition of cytoplasm of vegetative cell and generative cell is different. The generative cell is transparent and does not stain for RNA. The cytoplasm of the vegetative cell is rich in RNA. The nucleolus of the vegetative cell is larger and contains low amount of DNA. Its protein content increases after mitosis. The nucleus of the generative cell is small but contains high amount of DNA. It divides to form two sperm nuclei.

In some of the angiosperms, the development of the sperms occurs when the pollen is located within the anther but in others it happens when the anthers have dehisced. In most of the angiosperms, the pollen is released from the anther at the time when the generative cell is formed and sperm cells are produced after the pollen grains reach the stigma. In some of the plants, the division of the generative nucleus occurs in the pollen tube. Variations are noted in some plants. In some plants division takes place when pollen is still enclosed in the anther (*Portulaca oleracea*). In some plants this happens when the pollen has reached stigma, while in still others it happens when the pollen tube has emerged or when the pollen tube has reached the embryo sac. The division generally occurs by mitotic spindle and equatorial plate formation. A cell plate is formed. The partitioning takes place by formation of furrows (*Portulaca*).

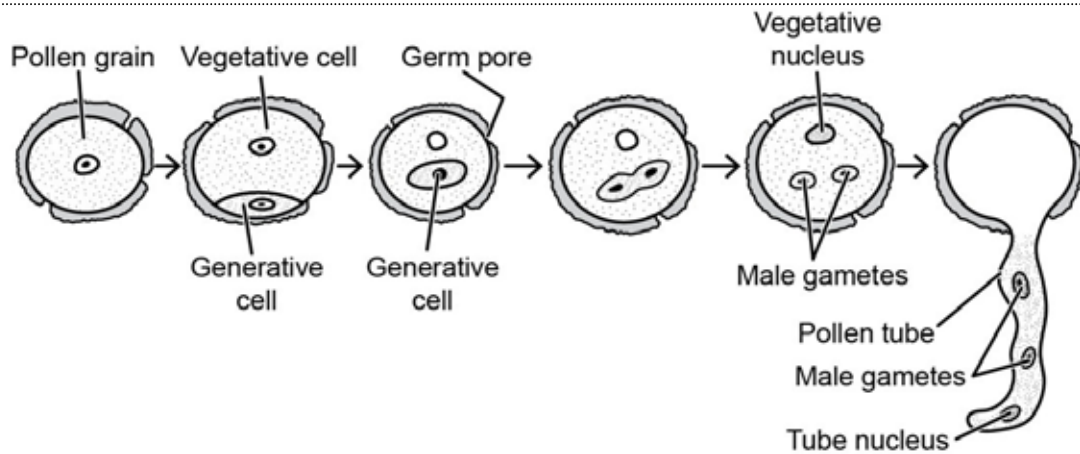


Fig. 9.10: Development of male gametophyte.

At the time of anther dehiscence if the pollen grains have undivided generative cell then these are described as binucleate. If sperms are already formed at this time then pollen is described as trinucleate.

9.5.1 Pollen Variants

In some plants, the male gametophyte forms variant structures. Of these we will discuss two examples.

In members of the family Cyperaceae, out of the four nuclei which are formed as a result of meiosis, only one functions, and the rest three degenerate. The functional nucleus remains in the centre and the rest are pushed towards the corner of MMC. The non-functional nuclei get separated from the functional nucleus by a wall. Walls are also formed between the non-functional nuclei. Thus from each MMC only one functional microspore is formed. The functional nucleus divides to form the vegetative and the generative cell.

9.5.2 Pollinia

The microspores generally get separated after meiosis in angiosperms. However, in some plants, they do not separate but form compound pollen grains. All the microspores in a microsporangium remain together and form a structure called **pollinium** (Fig. 9.11).

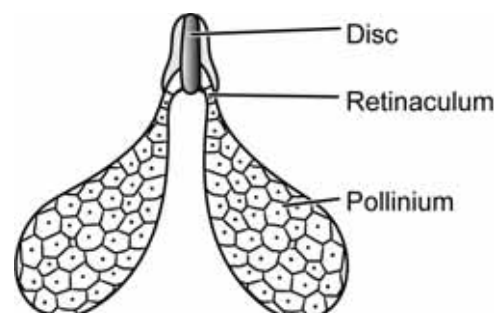


Fig. 9.11: Structures of Pollinia.

This has been commonly seen in the members of the family Orchidaceae and Asclepiadaceae. In some other plants the microspores in a tetrad undergo further division and produce polyads. The phenomenon is known as **polyspory**. In *Cuscuta reflexa* species, 11 microspores have been reported. In *Thunbergia mysorensis*, 22 microspores are present in a polyad.

SAQ 4

What are the different layers of pollen grain?

9.6 POLLEN STERILITY

Some plants lack the ability to produce or release functional pollen. This condition is referred to as male sterility. It results from the failure of formation or development of functional stamens, microspores or gametes. Male sterility can be classified as :

- a) Pollen sterility- when the pollen grains are sterile, mostly on account of meiotic abnormalities or tapetal disfunction;
- b) Structural or staminal male sterility- in this male flowers or stamens are malformed and non-functional or completely absent; and
- c) Functional male sterility- in which viable pollen grains are formed but do not get released because of indehiscent anthers.

Of the three types of male sterility, pollen sterility is the most common and plays a major role in plant breeding and hybrid seed production.

The main reason for the absence of pollen is breakdown of microsporogenesis which can be caused due to abnormal or malfunctioning tapetum. Based on its inheritance or origin, male sterility may be further divided into:

1. Nuclear male sterility (NMS) (genic, genetic or Mendelian sterility)- in this type, male sterility is governed by one or more nuclear genes;
2. Cytoplasmic male sterility (CMS) – in this type male sterility comes about as a result of the combined action of nuclear genes and genic or structural changes in the cytoplasmic organellar genomes (referred to as 'sterile cytoplasm') (S) as opposed to normal 'fertile cytoplasm' (N);
3. Non-genetic, chemically induced male sterility- these results from the application of specific chemicals referred to as gametocides or chemical hybridizing agents (CHA).

9.6.1 Nuclear Male Sterility

Nuclear male sterility (NMS) originates through spontaneous mutation. The frequency of such mutations is high. This has been noted in species like maize, tomato and barley. In these species, more than 40 non-allelic male sterility genes have been described. According to Duvick (1966), nuclear male sterility can probably be found in all diploid species. Spontaneous nuclear male sterility is usually controlled by a single recessive gene. But in some plants, it is found to be controlled by more than one recessive gene (Driscoll, 1986), by polygenes (Athwal *et al.*, 1967) or by dominant genes (Mathias, 1985).

9.6.2 Cytoplasmic Male Sterility

Cytoplasmic male sterility (CMS) may be divided into autoplasmic CMS and alloplasmic CMS based on its origin (Lacadena, 1968). Autoplasmic CMS

refers to the condition where the sterility has arisen within a species as a result of spontaneous mutational changes in the cytoplasm, most likely in the mitochondrial genome. Alloplasmic CMS refers to the condition where the male sterility has arisen from inter-generic, interspecific or occasionally intra-specific crosses. The male sterility can be interpreted as incompatibility or poor co-operation between the nuclear genome of one species and the organellar genome of another. Spontaneous autoplasmic CMS has been estimated to be only one quarter as frequent as spontaneous NMS (Kaul, 1988). The reason for this latter difference may be partly a greater genetic stability of the organellar genomes, partly the ability of nuclear genes to compensate for changes in the organellar genomes as evidenced by the occurrence of CMS restorer genotypes. The nuclear genetic control of CMS is predominantly governed by one or more recessive genes. Several theories have been presented for the cause of CMS. Experiments have shown that male sterility in *Petunia* is associated with a variant mitochondrial DNA arrangement (Evenor et al., 1988). The mitochondria have been regarded as the main site of the changes in the cytoplasm giving rise to CMS. This was first proposed by Rhoades (1950). Later on molecular techniques demonstrated that mitochondrial DNA (mtDNA) from fertile and male sterile cytoplasm in maize shows different restriction endonuclease digestion patterns. This strongly supported the hypothesis of a mitochondrial origin of CMS. Similar differences between the mtDNA from fertile and CMS plants have been found in many species including sugar beet, oilseed rape, sorghum, sunflower, rice and carrot.

9.7 STRUCTURE OF OVARY

The reproductive part of the flower bearing the ovules is called ovary. It continues into a cylindrical style that terminates in stigma. Ovary is attached to the flower through a basal stalk called stipe. Ovary that lacks stipe is considered sessile. The placement of floral parts namely hypanthium; calyx, corolla and androecium depend upon the position of ovary. An ovary is considered **superior** when the sepals, petals, stamens and/or hypanthium are attached at the base of the ovary and this condition is known as hypogynous. In case of an **inferior** ovary, sepals, petals, stamens and/or hypanthium are attached at the apex and thus surround ovary and this condition is known as epigynous. In some plants, intermediates between superior and inferior ovary are also found. In this case the sepals, petals, stamens and/or hypanthium are attached near the middle of the ovary and this condition is known as perigynous (Fig. 9.12). After post fertilization, the ovary is activated and forms the fruit.

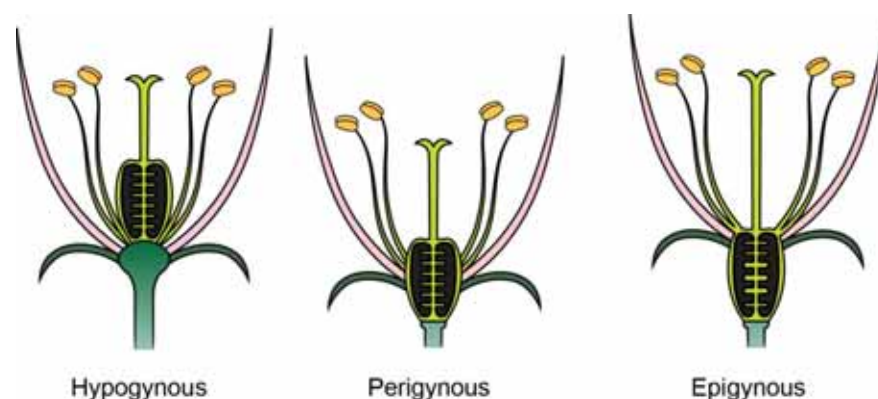


Fig.9.12: Type of ovaries with reference to their position.

9.7.1 Ovule

Ovule (megasporangium) is the structure which develop into seeds. It consists of a central mound of tissue known as **nucellus** which is enclosed by one or two coverings called **integuments**. A mature ovule consists of nucellar tissue enveloped by integuments leaving as small pore or opening at the apical end. The opening, called as **micropyle**, is the main passage for the entry of the pollen tube into the ovule. This end of the ovule is referred as the micropylar pole and the opposite pole where the funiculus is attached is called as the **chalazal pole**. The **funiculus** is the stalk like structure through which the ovule is attached to the placenta. The female gametophyte (embryo sac) is develops in the nucellus.

9.7.2 Structure of Ovule

Integuments - They form the covering of the ovule which matures to form the seed coat. The ovules can bear one integument (unitegmic condition) or two integuments (bitegmic). The bitegmic condition is common in Polypetalae and monocots, and unitegmic in sympetalae.

Nucellus - It is a homogenous mass of tissue present inside the integuments. This tissue differentiates to form the female gametophyte. During early stages of ovule development, an archaesporial cell differentiates in the hypodermal region of the nucellus. In some plants, the archaesporial cell functions as the megaspore mother cell (MMC). In this condition, the MMC remains hypodermal in position and gets surrounded by a single layer of nucellus in the micropylar region. Such ovules with minimal nucellus are called **tenuinucellate**. These are commonly seen in Sympetalae. In some plants, the hypodermal archaesporial cell undergoes periclinal division to form an outer primary parietal cell and inner primary sporogenous cell. The parietal cell remains undivided or it undergoes a few anticlinal and periclinal divisions to form several layers of cells above the sporogenous cell.

As a result of divisions of nucellar cells the sporogenous cell gets embedded inside a mass of nucellar tissue, such condition is called **crassinucellate**. In majority of the angiosperms, the nucellus is consumed by the growing embryo sac or the endosperm. In some species, it persists in the mature seed as the nutritive tissue. The persistent nucellus is called the **perisperm** (black pepper).

A few nucellar cells located at the base of the embryo sac but above the vascular supply of the funiculus becomes differentiated from adjacent cells. These cells may become sclerenchymatous, suberised or are thin walled and secretory. These cells form the **hypostase**. It is believed to function in

- transportation of nutrients
- act as a barrier or boundary for the growing embryo sac, it prevents embryo from growing at the base of the ovule.
- helps in maintaining the water balance in seed during dormancy
- produce certain enzymes or hormones and/or plays a protective role in mature seeds.

In some species, the cells of the nucellar tip become thick walled and differentiate to form **epistase**.

Endothelium - The nucellus degenerates during early stages of ovule development and embryo sac comes in contact with the innermost layer of the integument in the plants bearing unitegmic ovule. This layer of cells becomes specialized to supply nutrients to the embryo sac. The cells become radially elongated with cytoplasm rich in starch and lipids. The cell layer is referred as **endothelium**. Endothelium cells are similar to tapetal cells of anther as they store carbohydrates, protein, RNA and other metabolites. The cells are secretory in function, hence referred as integumentary tapetum.

Obturator - It is an outgrowth of placenta or funiculus or integument or style that projects toward the micropyle. It guides the pollen tube to the micropyle. The tissue is composed of thin walled cells. The cells show dense cytoplasm with high number of ER, dictyosomes and vesicles. The cells produce exudates and provide mechanical and chemical guidance to the growing pollen tube to enter the micropyle.

The ovule develops from specialized region in the ovary called placenta. Initially ovule looks like a mass of tissue on the placenta. It is composed of homogenous tissue. Later on it divides to form the integuments and undergo specific degree of curvature to form a mature ovule.

9.7.3 Types of Ovules

Ovules undergo specific degree of curvature during development in different plant species. The positions of the micropyle with respect to the funiculus become different (Fig.9.13). This distinguishes the ovules into different types. Ovules can be classified into six types-

1. **Anatropous** - This is the most common type of ovule found in angiosperms. In this type, the micropylar end comes and lies parallel and close to the funiculus.
2. **Orthotropous** - In this type, the micropyle faces upward away from the chalaza and lies straight in the same line with respect to funiculus.
3. **Campylotropous** - In this type, the ovule is curved but the curvature is much less than that in anatropous condition.
4. **Hemianatropous** - In this type, the micropyle comes and lies at right angles to the funiculus.
5. **Amphitropous** - In this type, the nucellus and the embryo sac become curved like a horse-shoe.
6. **Circinotropous** - In this type, the ovule first becomes anatropous then the curvature continues till the micropyle faces upward.

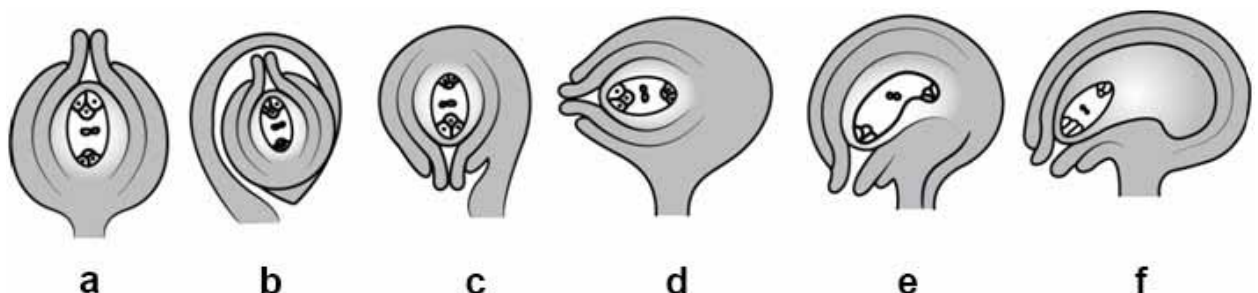


Fig. 9.13: Types of Ovules. a) Orthotropous; b) Circinotropous; c) Anatropous; d) Hemianatropous; e) Amphitropous; f) Campilotropous.

SAQ 5

- a) How is superior ovary different from inferior ovary?
- b) What is integumentary tapetum?

9.8 MEGASPOROGENESIS

The nucellar cells situated near the epidermis differentiate to form the archesporial cell. The cell is larger in size and possesses dense cytoplasm and prominent nucleus. In the tenuinucellate ovules this cell functions as the megaspore mother cell, while in the crassinucellate ovules, the archesporial cell divides transversely to form a primary parietal cell on the outer side and primary sporogenous cell on the inner side. The primary parietal cell may remain undivided or undergoes repeated divisions and pushes the sporogenous cell deeper in the nucellus. The primary sporogenous cell functions as the megaspore mother cell (megasporocyte). The cell possesses cellulosic cell walls and is connected to the neighbouring cells through plasmodesmata. It undergoes meiosis and forms four haploid megaspores. The deposition of callose takes place inside the cell wall of the megaspore mother cell. Due to callose deposition, the plasmodesmatal connections get closed. The nucleus of the megaspore mother cell undergoes meiosis followed by formation of transverse walls to form a dyad and then a tetrad of four megaspores. The four megaspores are generally arranged in a linear manner in the tetrad (Fig. 9.14).

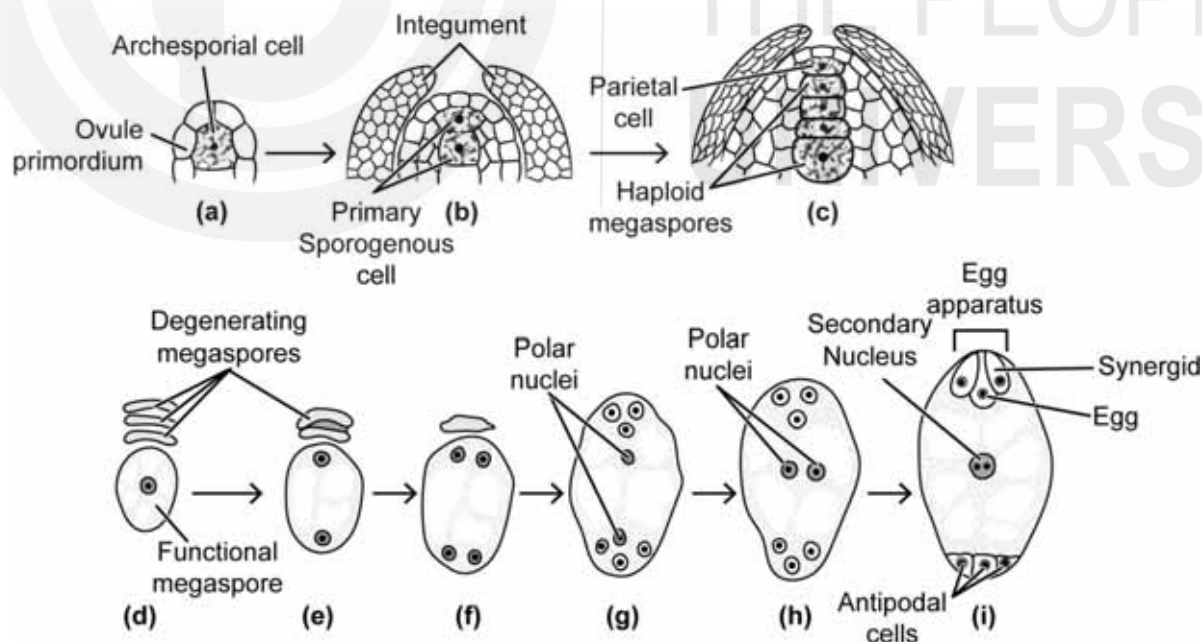


Fig. 9.14: Stages of Megasporogenesis.

Out of the four megaspores only one is functional and forms the female gametophyte or the embryo sac while the rest of three megaspores degenerate. In most plants, the megaspore located at the chalazal end is functional. In Polygonum type of embryo sac, the chalazal megaspore is functional.

During megasporogenesis, there is development of callose thickenings around the megasporocyte and the megaspores. The callose deposition first occurs at the chalazal region of MMC and then spreads over the entire surface separating it from the other maternal tissue. Callose thickenings first disappear from the functional megaspore.

9.9 STRUCTURE AND ORGANIZATION OF MATURE EMBRYO SAC (POLYGONUM TYPE)

The functional megaspore develops into a female gametophyte. Out of four, three megaspores degenerate while the fourth located close to the chalazal end develops into the female gametophyte. The development of gametophyte is initiated by enlargement of megaspore followed by three mitotic divisions to form a 7 celled 8 nucleate structure. The eight nuclei organize into egg apparatus with two Synergids and one egg, central cell with two polar nuclei and three antipodal cells. The polygonum type of embryo sac is the most common type in angiosperms.

9.9.1 Structure of Embryo Sac

The structure of mature embryo sac depicts a seven celled structure with three celled **egg apparatus** having two synergids and egg cell, a central cell having two polar nuclei that fuse to form polar nuclei as a secondary nucleus, and three antipodal nuclei/cells (Fig.9.15). The number and arrangement of nuclei/cells varies in mature embryo sacs of different types.

Some families such as Asteraceae and Ericaceae show some exceptions. Here the synergids project out into the micropyle. The egg cell show distinct polarity. The nucleus is embedded into the cytoplasm at the lower end while the upper end is vacuolated. Synergids are absent in *Plumbago* and *Plumbagella* types of embryo sacs. Here the egg cell takes the function of synergids too. The antipodals are absent in *Oenothera* type. The cells of egg apparatus and antipodals are uninucleate and haploid, while the central cell is binucleate or diploid. The embryo sac remains confined to nucellus during its growth and maturation. A multilayered embryo sac wall is seen in maize while a wall rich in pectic substances is noted in cotton. The inner wall of female gametophyte represents functional wall of the megaspore while the outer layers are remains of the crushed nucellus. In some plants, embryo sac at the micropylar and nucellar end develops extensions (finger like outgrowths). This has been noted in plants of *Acanthaceae*, *Santalaceae*, *Stylidiaceae* and many others.

Synergids - These are the elongated cells with tip prolonged into a beak like structure. The cell wall is thick in the micropylar region, thin in the centre and finally disappears. They show a characteristic feature called filiform apparatus at the micropylar end. The organelles (mitochondria, dictyosomes, endoplasmic reticulum) occupy a region between the nucleus and filiform apparatus in the micropylar region of the cell. The pollen tube enters the embryo sac by penetrating one of them which usually degenerates before or immediately after arrival of the pollen tube. The synergids provide nutrition and chemical attractant to the pollen tube. In *Plumbago* where synergids are

absent the pollen tube enters directly into the embryo sac through the egg cell. The egg cell in this type possesses the filiform apparatus. These functions of synergid are performed by the egg cell itself. The synergids are ephemeral but play an important role during fertilization by secreting chemotropic substances that guide the growth of the pollen tube. In plants belonging to Compositae, Crassulaceae, Santalaceae, and Poaceae the synergids are haustorial in nature.

Antipodals - The cells exhibit maximal variation in number, size, ploidy level and may be ephemeral or persistent. In most of the plants three antipodal cells are present but in some plants they undergo secondary multiplication and may become polyploid or become polytenic and multinucleate. In *Drusa*, 11 antipodals are present. If the walls are not formed after division, the cells become multinucleate as in family Dipsacaceae. The antipodal cells become polyploid due to endomitosis. In rare cases, such as in *Aconitum napellus* the cells enlarge and the tip reaches the egg apparatus. Some of these cells develop papillate like wall projections into the cytoplasm (poppy and rice). The antipodals are rich in ascorbic acid, starch, lipids and proteins. They show low concentration of RNA and polysaccharides. They also show haustorial behaviour in some plants (*Quinchamalium*).

Central cell - The central cell is separated from the egg cell and synergids by the plasma membrane and from antipodals by a thin wall traversed by plasmodesmata. The outer wall shows ingrowths that help in the absorption and transport of nutrients. The cell possess two polar nuclei in Polygonum type embryo sac, but *Oenothera*, *Paeonia*, *Plumbago*, *Peperomia* and *Chrysanthemum* types the cell has more than two nuclei, all nuclei fuse together to form a secondary nucleus (polyploid).

Female gamete (Egg) - It is present associated with synergids which together form the egg apparatus. The wall of the egg is thicker towards the micropyle and thinner towards the chalazal side. The cell is traversed by plasmodesmata on the wall adjoining the two synergids and the central cell (Fig. 9.15).

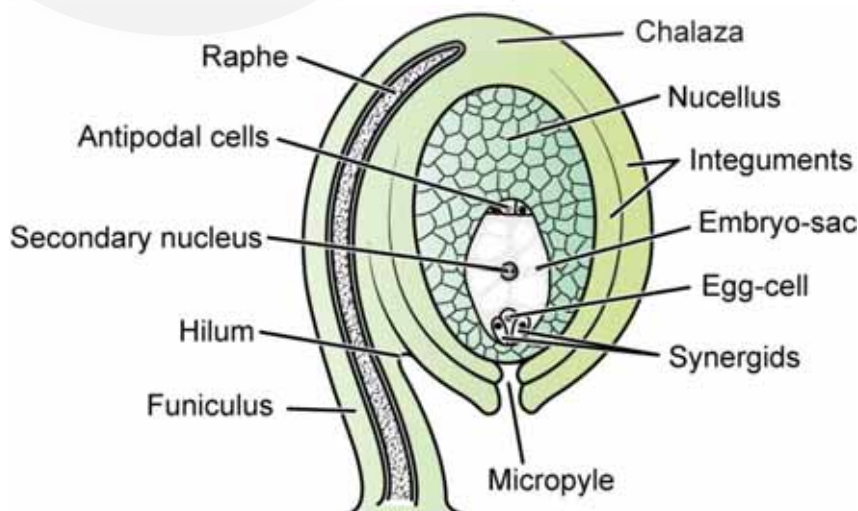


Fig. 9.15: Structure of embryo sac.

It shows the presence of high amount of plastids, starch content is abundant around the nucleus. In *Plumbago* finger like wall projections similar to filiform apparatus of synergids arise at the micropylar end of the egg cell.

9.9.2 Development of Embryo Sac

Depending upon the number of megaspore nuclei involved in the formation of the gametophyte, the embryo sac development has been classified into different types. These include monosporic, bisporic and tetrasporic types. Each type of embryo sac has more than one variant and is generally named after the genus (Fig. 9.16).

Monosporic

The basal megaspore of the tetrad undergoes three mitotic divisions. The resulting nuclei differentiate into a three celled egg apparatus at the micropylar pole, two polar nuclei in the central cell and three antipodal cells at the chalazal pole. This represents eight nucleate Polygonum type of embryo sac. If the megaspore undergoes two mitotic divisions, the embryo sac is four nucleate having three celled egg apparatus and one polar nucleus. This represents Oenothera type of embryo sac.

In Polygonum type, the development of embryo sac begins with the elongation of the functional megaspore. The cytoplasm in this megaspore is non-vacuolate but later vacuoles appear which fuse to form a large vacuole in the centre. The wall is not formed after the division of the nucleus. The large vacuole expands and pushes the nuclei towards the opposite poles of the cell. Both nuclei divide and form four nuclei with two at each pole. Further division leads to the formation of the eight nucleate structure. The cellular organization takes place. Out of the four nuclei located at the micropylar end, three organize into egg apparatus. The fourth nucleus remains free and acts as the upper polar nucleus. Three nuclei at the chalazal side form the three antipodal cells and the fourth one function as the lower polar nucleus.

In Oenothera type, the embryo sac is derived from the micropylar megaspore of the tetrad and is four nucleate. The mature embryo sac has an egg apparatus and a uninucleate central cell.

Bisporic

The embryo sac is derived from one of the two dyads (the lower dyad cell) produced after first meiotic division. The other dyad subsequently degenerates. The cell wall formation does not occur in the dyad after the second division and the haploid nuclei undergo two mitotic divisions to form eight nuclei. The final organization of the embryo sac is similar to Polygonum type. In case the upper (micropylar) dyad cell develops into an eight nucleate embryo sac it is called Endymion type, while in plants where the lower (chalazal) dyad cell develops into an eight nucleate embryo sac it is called Allium type.

Tetrasporic

In this case wall is not formed after meiosis so all the four haploid nuclei remain in the common cytoplasm forming a **coenomegaspore**. The arrangement of four megaspore nuclei determines the type of tetrasporic embryo sac.

A 2 micropylar and 2 chalaza arrangement of haploid nuclei followed by mitotic division of each produces eight nuclei which organize like Polygonum type of embryo sac.

A 1+1+1+1 disposition of haploid nucleus is followed by one mitotic division and out of the eight nuclei, four nuclei migrate to the to the centre and function as polars, remaining four organize as the egg cell, antipodal cell and one each of the lateral cells- Plumbago type.

Female Gametophyte type	Megasporogenesis			Megagametogenesis			
	Mega-sporocyte	Meiosis I	Meiosis II	Meiosis I	Meiosis II	Meiosis III	Mature female gametophyte
Monosporic 8-nucleate <i>Polygonium</i> type							
Monosporic 4-nucleate <i>Oenothera</i> type							
Bisporic 8-nucleate <i>Allium</i> type							
Tetrasporic 16-nucleate <i>Peperomia</i> type							
Tetrasporic 16-nucleate <i>Penaea</i> type							
Tetrasporic 16-nucleate <i>Drusa</i> type							
Tetrasporic 8-nucleate <i>Fritillaria</i> type							
Tetrasporic 8-nucleate <i>Plumbagella</i> type							
Tetrasporic 8-nucleate <i>Plumbago</i> type							
Tetrasporic 8-nucleate <i>Adoxa</i> type							

Fig. 9.16: Types of embryo sac (After Simpson).

If four haploid nuclei divide and undergo two mitotic divisions, the resulting 16 nuclei organize into four groups. The upper three cells on egg apparatus, two lateral of three cells each, four nuclei function as polars- Panaea type.

The 16 nuclei can also organize as two celled egg apparatus, six antipodal cells, and eight polar nuclei- Peperomia type.

A 1+3 disposition of haploid nucleus is followed by two mitotic divisions, and 16 nuclei arrange as three- celled egg apparatus, two polars and 11 antipodal cells- Drusa type.

A 1+3 haploid nucleus undergoes two mitotic divisions. During the first division, three mitotic spindles at chalazal end fuse. The second mitotic division results in the formation of four haploid micropylar and four triploid chalazal nuclei. Three haploid nuclei form the egg apparatus, one haploid and one triploid nucleus function as polars, and three triploid nuclei as antipodals- Fritillaria type.

A 1+2+1 arrangement of megaspore nuclei, the micropylar and chalazal nuclei divide twice. The embryo sac comprises of three celled egg apparatus, four antipodal cells, and three polar nuclei. If the two central nuclei fuse and micropylar and chalazal nuclei undergo two mitotic divisions, the embryo sac comprises of three celled egg apparatus, one haploid and one diploid nucleus which together function as polars, remaining three haploids and three diploids organize as antipodals - *Chrysanthemum cinerariifolium* type. The embryo sac lacks synergids in Plumbago and Plumbagella type, while one synergid is found in Pepromia type.

In some species, more than one types of embryo sac is found. In *Erigeron sp.* all the three types viz., mono, bi and tetrasporic type of embryo sacs are found

9.9.3 Haustorial Behavior of Embryo Sac

The central cell forms multicellular projections from micropylar or chalazal end. These projections penetrate the carpellary tissues and function as haustoria. In Loranthaceae, the embryo sac is elongated. The chalazal end is the main route for entry of nutrients. The nutrients reach the embryo sac via nucellus. A specialized tissue called hypostase is present between the funicular vascular supply and the chalazal end of the embryo sac. In some cases nucellar tissue is consumed during development of embryo sac. Inner integument becomes glandular epithelium and absorbs nutrients from outer tissues and supplies them to embryo sac. Since endothelium is structurally and functionally similar to anther tapetum, it is called as **integumentary tapetum**.

SAQ 6

- a) Synergids are ephemeral in nature but form an important component of embryo sac. Explain.
- b) Enumerate two types of bisporic embryo sacs.
- c) Fill in the blanks:
 - i) Flower bears ovaries enclosed inside the.....
 - ii) The flower bearing only stamens are referred as
 - iii) The fusion of various floral appendages is called as
 - iv) The development of flower starts from the
 - v) The flower organization in plants is regulated by
 - vi) Early anther development starts with the differentiation of

- vii) acts as the nutritive tissue that helps in the development and maturation of microspores.
- viii) layer of pollen is composed of sporopollenin.
- ix) a small lens shaped cell present in the male gamete that subsequently divides to form two sperm cells (microgametes).
- d) Choose the correct option for the given statements:
- i) In this structure the microspores in a microsporangium remain together.
- a) anther
- b) pollinium
- c) syncytium
- ii) Embryo sac is a nucleate structure.
- a) 6
- b) 8
- c) 10
- iii) The process of development of microspores from microspore mother cells is referred as:
- a) gametogenesis
- b) spermatogenesis
- c) microsporogenesis
- iv) During flower development, this initial gives rise to lateral flower buds and bracts.
- a) *annaeu*
- b) *meristem d'attente*
- c) mantle
- v) In which type of embryo sac, the development begins with the elongation of the functional megaspore.
- a) Oenothera
- b) Peperomia
- c) Polygonum type
- vi) In this arrangement of microspores in a tetrad, the nuclei are arranged one above the another.
- a) isobilateral
- b) tetrahedral
- c) linear

9.10 SUMMARY

- A flower is structure bearing reproductive parts in plants and evolved after structural modification in shoot apex.
- A flower generally bears four whorls, namely sepals, petals, stamens and carpels which form the calyx, corolla, androecium and gynoecium. A flower bearing all the four whorls or appendages is called as complete flower.
- The histological zonation of vegetative apex helps in the development of different parts of the flower. Whole apex gets transformed to enter new phase of development. The cells present in different zones of reproductive apex show high mitotic activity and result in formation of different parts of the flower. Cells located in the tunica and mantle region undergo divisions followed by differentiation to form different parts of the flower.
- The basic organization of flowers is explained by ABC model. Three classes of genes regulate the development of floral organs in angiosperms. The interaction between these genes induces the development of floral organs.
- Anther is the structure that produces pollen. A mature anther is a four lobed structure having four pollen sacs. A mature anther wall is made up of different layers. Outer epidermis is followed by endothecium, middle layers and tapetum. The endothecium helps in the dehiscence of the anther, while tapetum acts as the nutritive tissue that supports the developing pollen.
- A group of archaesporial cells present in the anther lobe undergo periclinal division to form primary parietal cell (PPC) and primary sporogenous cell (PSC). The PPCs undergo repeated periclinal and anticlinal divisions to produce 2 to 5 layers of the anther wall. The primary sporogenous cells undergo mitotic division to form microspore mother cells (MMC) or microsporocytes which further undergo meiosis to produce four microspores. The process of development of microspores from microspore mother cells is referred as microsporogenesis.
- Microspores develop into the male gametophyte or microgametophytes. Each male gametophyte contains three cells located within the cell wall. The microspore nucleus divides mitotically to produce a large **vegetative** cell and a small lens- shaped **generative** cell which subsequently divides further to form two sperm cells (microgametes).
- Some plants show inability to produce or to release functional pollen. This results from failure of formation or development of functional stamens, microspores or gametes. Male sterility is also designated as nuclear male sterility (NMS) (genetic or Mendelian sterility) which is governed by one or more nuclear genes, or cytoplasmic male sterility (CMS) in which the sterility results from combined action of nuclear genes and genic or structural changes in the cytoplasmic organellar genomes. Non-genetic male sterility results from the application of specific chemicals (gametocides) or chemical hybridizing agents (CHA).

- Ovary is the reproductive structure in flower that bears the ovules which bear the female gametophyte. It has a cylindrical structure called style that terminates into stigma.
- Ovule or megasporangium is the structure that develops into seeds. It consists of a central mass of tissue known as nucellus enclosed by one or two integuments.
- The region of ovule from where the pollen tube enters the ovule is the micropylar pole and the opposite end where the funiculus (stalk through which ovule is attached to placenta) is present is called as the chalazal pole.
- Ovules undergo specific degree of curvature during development, therefore the position of the micropyle with respect to the funiculus becomes different. On the basis of this, ovules are classified into different types: anatropous, orthotropous, campylotropous, hemianatropous, amphitropous and circinotropous.
- The process of development of megaspore which subsequently develops into the female gametophyte or the embryo sac is called megasporogenesis.
- The mature embryo sac is eight celled structure having three celled egg apparatus, three antipodal nuclei/cells and two polar nuclei or fused polar nuclei as a secondary nucleus.
- The embryo sac development has been classified into three types: monosporic, bisporic and tetrasporic depending upon the number of megaspore nuclei involved in the formation of the gametophyte.

9.11 TERMINAL QUESTIONS

1. Differentiate between :
 - i) complete and incomplete flower
 - ii) gamosepalous and polysepalous flower
 - iii) monadelphous and polyadelphous stamens
 - iv) secretory and amoeboid tapetum
 - v) exine and intine
2. Which is the most active layer of anther wall and why?
3. How is nuclear male sterility different from cytoplasmic male sterility?
4. Explain the structure of male gametophyte.
5. What is pollinium?
6. Describe in detail the structure of a *Polygonum* type of embryo sac.
7. Fill in the blanks with the appropriate word:
 - i) Synergids are absent in and types of embryo sac.

- ii) The antipodals are absent in type of embryo sac.
 - iii) The finger like wall projections in synergids forms the apparatus.
 - iv) Ovary is attached to the flower through a basal stalk called
8. Write short note on importance of:
- i) nucellus
 - ii) endothelium
 - iii) hypostase
9. Differentiate between tenuinucellate and crassinucellate ovules.

9.12 ANSWERS

Self-Assessment Questions

1. a) **Calyx** - Sepals that form the outermost whorl of flower are together referred as calyx. They are modified leaves which enclose the other flower parts and are mostly green in colour. They are the thickest, toughest part of the flower and protect the bud as it develops into flower. Sepals in some flowers are fused to form gamosepalous calyx.

Corolla - Petals present inside the calyx form the corolla. They are generally large, broad, thin flat, brightly coloured, showy. They may be free or fused to form a tube like structure. Petals in some flowers are fused to represent gamopetalous corolla. They attract insects which help in pollination.

Androecium - Stamens enclosed within the inner whorl of corolla form the androecium. The stamens may occur free, in whorls of one or two or fused among themselves or with petals. When stamens are fused to form one group, the condition is called as **monoadelphous**. Stamens fuse laterally to form a stamina tube. When stamens are fused to form several groups, the condition is called as **polyadelphous**. In some flowers stamens may be reduced or sterile, called as staminodes. Each stamen bears a filament that bears anther. Each anther is a four lobed structure having pollen grains. Each pollen grain produces two male gametes or sperms.

Gynoecium - Carpels located in the middle of the flower terminating the flowering shoot is referred as gynoecium. A flower can have one carpel or two or more carpels which can be free or fused. The gynoecium or pistil generally possesses three parts namely- ovary, style and stigma. Ovary encloses ovules which bear the female gametophyte.

- b) The flower in which only stamens are present is referred as staminate flowers, while those in which only carpels are present are called carpellate or pistillate flowers.
2. a) According to tunica corpus organization, the meristematic mantle gives rise to floral parts and contributes cells to parenchymatous core inside. The tunica-corporis organization exists in the reproductive apex. The vegetative stem apex gets transformed to reproductive apex. The reproductive apex develops certain changes and becomes modified. The apex becomes more elongated and conical and the frequency of mitotic divisions and DNA synthesis increases. The cells of the central core of the apex become highly vacuolated. The smaller cells form the outer covering or mantle. The floral apex develops a mantle-core organization. The reproductive apices retain cyto-histological zonation of the vegetative apex. Plantefol (1947) gave the opinion that reproductive apex originates from transformation of vegetative apex. The anneau initial gives rise to foliage leaves in the vegetative phase and produces sepals, petals in the reproductive phase. Stamens and carpels are derived from residuum of the apex. The sepals are derived from anneau initial where as other floral appendages are initiated in the meristematic mantle which is derived from *meristeme d'attente*. The *meristeme d'attente* is responsible for generation of bracts and flowers.

Many authors suggest that whole apex gets transformed to enter new phase of development. The vegetative apex helps in the promotion of longitudinal extension of the axis, while the reproductive apex helps in the development of different parts of the flower.

- b) The basic organisation of flowers is explained by ABC model. According to this model interaction between three classes of genes regulate the development of floral organs in angiosperm. Three classes of genes namely A, B and C control the basic aspects of flower organization. Gene A affects the outermost, lowest regions of the flower shoot apical meristem, gene C controls the uppermost, central region and gene B overlaps the other two. In the region where gene A is expressed, the primordial develops into sepals. The primordium in the upward and inward region is influenced by genes A, B and they develop into petals. The combination of genes B and C produces stamens, and in the center of the flower where gene C is expressed primordial develop into carpels. This means that class A genes when expressed induce the development of sepals (whorl 1). Interaction between class A and class B genes induce the development of petals (whorl 2). Stamens are formed as a result of interaction between class B and class C genes (whorl 3). Class C gene induces the formation of carpel (whorl 4). Class A genes together and class C gene alone are responsible for the development of sepals and carpel respectively. The class B genes and class A genes function cooperatively to determine the development of petals. The ABC model was formulated by Coen et al. in 1991.

3. A mature anther wall is made up of different layers. The outermost layer is the epidermis followed by endothecium, middle layers and tapetum.

The endothecium layer helps in the dehiscence of the anther. It is usually single layered. The cells are radially elongated, get enlarged and develop fibrous thickenings which help in dehiscence of anther to discharge pollen grains.

Inner to endothecium lie one to three layers of cells which form the middle layers. These layers originate from PPCs. the cells of the middle layers are generally rich in reserve food such as starch. The cells get collapsed due to expansion of endothecium and tapetum.

Tapetum forms the innermost layer that surrounds the sporogenous tissue. Tapetum cells consist of metabolically active cells. It acts as the nutritive tissue that helps in the development and maturation of microspores.

4. The pollen wall is made up of two layers. The inner layer is called intine which is made up of pectocellulose. This layer gets degraded during acetolysis. Intine is also differentiated into two layers. The outer pectic polysaccharide layer called exintine and the inner cellulosic layer called endintine respectively. The outer pollen layer is called exine. This layer is resistant to acetolysis and physical and chemical degradation. Exine is composed of sporopollenin which is a highly resistant material derived from carotenoids after polymerization. Exine is differentiated into two layers- outer sculptured layer, the sexine and the inner non-sculptured layer-nexine.

5. a) An ovary is considered superior when the sepals, petals, stamens and/or hypanthium are attached at the base of the ovary. In case of an inferior ovary, the sepals, petals, stamens and/or hypanthium are attached at the apex of the ovary.
- b) Inner integument becomes glandular epithelium and absorbs nutrients from outer tissues and supplies them to embryo sac. Since endothelium is structurally and functionally similar to anther tapetum, it is called as integumentary tapetum.
6. a) The synergids are ephemeral but play an important role during double fertilization because they secrete chemotropic substances that guide the direction of the growth of the pollen tube. In plants belonging to Asteraceae, Crassulaceae, Santalaceae and Poaceae the synergids are haustorial in nature. The filiform apparatus aids in the absorption and transportation of materials into the embryo sac from the nucellus. It is also site of entry of pollen tube in one of the synergids.
- b) Bisporic embryo sac is derived from the lower dyad produced after first meiotic division. After the second division the cell wall formation does not take place in the dyad. The haploid nuclei undergo two mitotic divisions to form eight nuclei. It is categorised into two types :

Endymion type - in this upper (micropylar) dyad cell develops into an eight nucleate embryo sac.

Allium type - in this the lower (chalazal) dyad cell develops into an eight nucleate embryo sac.

- c)
 - i) Carpels
 - ii) Staminate flowers
 - iii) Adnation
 - iv) histological zonation of vegetative apex
 - v) temporal or spatial pattern of homeotic genes
 - vi) archaesporial cells
 - vii) tapetum
 - viii) exine
 - ix) generative
- d)
 - i) b. pollinium
 - ii) b. 8
 - iii) c. microsporogenesis
 - iv) a. *annaui*
 - v) c. Polygonum type
 - vi) c. linear

Terminal Questions

1.
 - i) complete and incomplete flower - A flower having all the four whorls namely- calyx, corolla, androecium and gynoecium is referred as complete flower while that lacking any one of the four whorls is called as incomplete flower.
 - ii) gamosepalous and polysepalous flower - Flowers in which sepals are fused are called as gamosepalous, while the flower in which sepals are free are called as polysepalous.
 - iii) monadelphous and polyadelphous stamens-When stamens are fused to form one group, the condition is called as monadelphous (example- *Hibiscus*). Stamens fuse laterally to form a staminal tube. When stamens are fused to form several groups, the condition is called as polyadelphous.
 - iv) secretory and amoeboid tapetum - In amoeboid tapetum (invasive or periplasmopodial) inner tangential and radial walls breakdown, protoplasts enlarge and their fusion results in the plasmodium. Amoeboid tapetum forms the periplasmodium. The amoeboid tapetum is found commonly in monocotyledons. The secretory or

glandular (parietal or cellular) type of tapetum remains intact throughout the microspore development and provides nutrition to the developing microspores. It is commonly found in dicotyledons. This type of tapetum degenerates *in situ* at later stages of microspore development.

- v) exine and intine - The inner pectocellulosic layer of the pollen grain is called as intine which gets degraded during acetolysis. Intine is also differentiated into two layers. The outer pectic polysaccharide layer called exintine and the inner cellulosic layer called endintine respectively. The outer layer of the pollen wall is called exine which is resistant to acetolysis and physical and chemical degradation. Exine is composed of sporopollenin which is a highly resistant material derived from carotenoids after polymerization. Exine is differentiated into two layers- outer sculptured layer, the sexine and the inner non-sculptured layer- nexine.

2. Tapetum is the most active layer of the anther wall. It synthesizes certain substances such as fibro-granular proteins, and carotenoid containing lipid droplets which get released in the anther locule during maturation of pollen grains.

3. Nuclear male sterility (NMS) (genic, genetic or Mendelian sterility) - in this type, male sterility is governed by one or more nuclear genes. This type of sterility originates through spontaneous mutation. This has been noted in species like maize, tomato and barley. Spontaneous nuclear male sterility is usually controlled by a single recessive gene.

Cytoplasmic male sterility (CMS) - in this type male sterility comes about as a result of the combined action of nuclear genes and genic or structural changes in the cytoplasmic organellar genomes (referred to as 'sterile cytoplasm') (S) as opposed to normal 'fertile cytoplasm' (N). This type of sterility may be autoplasmic or alloplasmic. Autoplasmic CMS refer to that where the sterility has arisen within a species as a result of spontaneous mutational changes in the cytoplasm, most likely in the mitochondrial genome. Alloplasmic CMS, refers to condition where the male sterility has arisen from inter-generic, interspecific or occasionally intra-specific crosses. The nuclear genetic control of CMS is predominantly governed by one or more recessive genes. CMS have been found in species including sugar beet, oilseed rape, sorghum, petunia, sunflower, rice and carrot.

4. Microspores develop into microgametophytes. In all the angiosperms, the male gametophyte or microgametophyte is small and consists of three cells located within the cell wall. The microspore nucleus migrates to the side of the pollen grain and divides mitotically to produce a large vegetative cell and a small lens-shaped generative cell which subsequently divides further to form two sperms cells (microgametes). In some of the angiosperms, the developments of the sperms occur when the pollen is located within the anther. In most of the angiosperms, the pollen is released from the anther at the time when the generative cell has formed and sperms cells are produced after they reach the stigma or in the pollen tube.

5. The microspores get separated after meiosis. However in some plants, they do not separate but form compound pollen grains. When the microspores in a microsporangium remain together, they form a structure called pollinium. These are found in members of families Orchidaceae and Asclepiadaceae.
6. The structure of mature embryo sac depicts a seven celled structure with three celled egg apparatus having two synergids and egg cell, a central cell having two polar nuclei or fused polar nuclei as a secondary nucleus, and three antipodal cells. The number and arrangement of nuclei/cells varies in mature embryo sacs.

Synergids are the elongated cells. The cell wall is thick in the micropylar region, membranous in the centre and finally disappears. They show a characteristic feature called filiform apparatus at the tip. When synergids are present, the pollen tube enters through one of them, but if they are absent the pollen tube enters directly into the embryo sac through the egg cell. The synergids provide nutrition and chemical attractant to the pollen tube. In cells in which the synergids are absent, these functions are performed by the egg cell. The synergids are ephemeral but play an important role during fertilization by secreting chemotropic substances that guide the growth of the pollen tube.

Antipodal cells exhibit maximal variation in number, size, ploidy level and may be ephemeral or persistent. In some plants, they undergo secondary multiplication and may become polyploid due to endomitosis, or become polytenic and multinucleate. Some of these cells develop papillate wall projections into the cytoplasm. The antipodals are rich in ascorbic acid, starch, lipids and proteins. The central cell is separated from the egg cell and synergids by the plasma membrane. The outer wall shows ingrowths that help in the absorption and transport of nutrients. The cell generally possesses two polar nuclei. The nuclei fuse together to form a secondary nucleus (polyploid).

Female gamete (Egg) is present associated with synergids which form the egg apparatus. The wall of the egg is thicker towards the micropyle and thinner towards the chalazal side. It shows the presence of high amount of plastids, starch content present abundantly around the nucleus. In some cases such as *Plumbago* type, finger like wall projections arise at the micropylar end of the egg cell.

7.
 - i) *Plumbago* and *Plumbagella*
 - ii) *Oenothera*
 - iii) filiform
 - iv) stipe
8.
 - i) **Nucellus** - It is a homogenous mass of tissue present inside the integuments. This tissue differentiates to form the female gametophyte. The archaesporial cells differentiate from the hypodermal region of the nucellus. In some plants, the hypodermal archaesporial cells undergo periclinal division to form an outer parietal cell and inner sporogenous cell. The parietal cells remain undivided or undergo few anticlinal and periclinal divisions to form several layers of cells above the sporogenous tissue.

- ii) **Endothelium** - The cells of the innermost layer of the integument become specialized to supply nutrients to the embryo sac. The cells become radially elongated with cytoplasm rich in starch and lipids. The layer of cells is also referred as integumentary tapetum.
 - iii) **Hypostase** - A few nucellar cells located at the base of the embryo sac become sclerenchymatous, suberised or thin walled and secretory to form the hypostase. These cells help in transportation of nutrients, act as a barrier for the growing embryo sac, it prevents embryo from growing at the base of the ovule, help in maintaining the water balance in seed during dormancy and produce certain enzymes or hormones and/or plays a protective role in mature seeds.
9. The ovules in which the sporogenous tissue remains hypodermal in position and gets surrounded by a single layer of nucellus in the micropylar region are called **tenuinucellate**. These are commonly seen in Sympetalae. The hypodermal archesporial cells undergo periclinal division to form an outer parietal cell and inner sporogenous cell. The ovules in which the nucellar cells situated above the sporogenous tissue divide to form layers of cells, so that the sporogenous cell gets embedded in the mass of nucellar tissue. Such condition is called **Crassinucellate**.

UNIT 10

POLLINATION|

Structure

10.1	Introduction	10.3	Pollination Mechanisms
	Objectives		Anemophily
10.2	Pollination		Hydrophily
	Types of Pollination		Entomophily
	Self- Pollination		Ornithophily
	Advantages and Disadvantages of Self-Pollination		Cheiropterophily
	Cross-Pollination		Zoophily
	Advantages and Disadvantages of Cross Pollination	10.4	Pollen - Stigma Interaction
			Wet and Dry Stigma
			Open and Closed Style
		10.5	Summary
		10.6	Terminal Questions
		10.7	Answers

10.1 INTRODUCTION

In the earlier unit 9 you have studied about the structure and development of male and female gametophytes. The male gametes are produced via process of microsporogenesis, while female gametes are formed after megasporogenesis. After the formation of the gametophytes, the process of sexual reproduction involves the process of double fertilization in which the fusion of one of the male gametes with the egg takes place to form the zygote which develops into embryo. Second male gamete fuses with the central cell nuclei to form a nutritive tissue called the endosperm. Before fertilization, pollen needs to be transferred from the stamen to the stigma of the carpel. The transfer of pollen is known as **pollination**. The transfer of pollen takes place with the help of various agents such as wind, water or animals. In this unit you will become familiar with different types of pollination, some of the important adaptation exhibited by plants for successful pollination, about the various agents involved in pollination.

Objectives

After studying this unit you should be able to :

- ❖ explain the process of sexual reproduction in plants;
- ❖ describe the methods the plants adapt to disperse the pollen grains for effective pollination ;various types of pollination; and
- ❖ explain the reactions of pollen pistil interaction which relate the control mechanism that plants developed to avoid indiscriminate sexual reproduction.

10.2 POLLINATION

The pollen grains germinate when they are transferred to stigma of a flower on same or another plant of the same species. Thus pollination refers to the transfer of pollen from dehiscing anthers to the pistil or the process of transfer of pollen from anther (male reproductive part) to the stigma (female reproductive part) of the same flower or different flower is called as pollination.

As you know that plants cannot move to their mates for sexual reproduction hence the transfer of pollen grains from male parent to stigma of the female parent takes place with the help of certain agents. The process helps in transferring the male gamete to the female reproductive structure, thereby facilitating the fertilization to produce the offspring.

Anther dehiscence; It simply means the release of pollen grains from dry and mature anthers. The changes in the environmental conditions create an external pressure that leads to the rupture of the anther wall by mechanical pressure resulting in release of mature pollen from the anther sac. The breakage of the anther wall is supported by endothelial cells present along the stomium. The hygroscopic nature and differential expansion of tangential walls of the endothelial cells play a role in dehiscence of anthers. In most of the angiosperm the stomium is a narrow strip along the entire length of the anther lobe. It may however, also be restricted to a lid or valve (*Berberidaceae*) or pores (*Solanum*, *Cassia*, *Polygala*). Stigmas, the receptive portions of the carpel, bind pollen of the same species and mediate tube growth and passage migration into the style and ovary.

10.2.1 Types of Pollination

Depending on the source of the pollen, pollination is of two types:

1. **Self-pollination** - This involves the transfer of pollen from anther to stigma of the same flower or of another flower on the same plant. This can occur in the unisexual or bisexual flowers which occur on the same individual plant.
2. **Cross-pollination** - This involves the transfer of pollen from one flower to the stigma of a flower on another plant of the same species. It can occur in both monoecious and dioecious plants.

10.2.2 Self-Pollination

This process involves the transfer of pollen from the same flower or another flower on the same plant (Fig. 10.1). The self-pollination can be of different types:

- i) **Autogamy** - In this type of pollination, the pollen grains of an anther is transferred to the stigma of the same flower. This means pollination of flower by its own pollen. The stigma and anthers of flowers ripe simultaneously and are positioned in such a manner that pollen grains can be easily transferred to own stigma. This type of pollination is found in members of Apiaceae and Cactaceae. **Cleistogamy** is a condition in which the flowers do not open but pollination occurs within a closed flower. In cleistogamous flowers, the some peculiar structural adaptation has been noted. These include underground bisexual flowers which never open (*Commelina bengalensis*, *Viola*, *Oxalis*, *Juncus* and *Saxifraga*). Thus, self-pollination is seen in both cleistogamous flowers (which remain closed) and chasmogamous flowers (which are open).
- ii) **Geitonogamy** - In this condition, the pollen of a flower pollinate any other flower present on the same plant. This type of pollination occurs between two different flowers present on the same plant. Flowers in this type also show adaptations such as **homogamy**- a condition in which anthers and stigma of a flower mature at the same time (*Mirabilis jalapa*, *Argemone mexicana* and *Tagetes indica*). Since all flowers of a plant are genetically similar, geitonogamy is regarded a form of self-pollination.

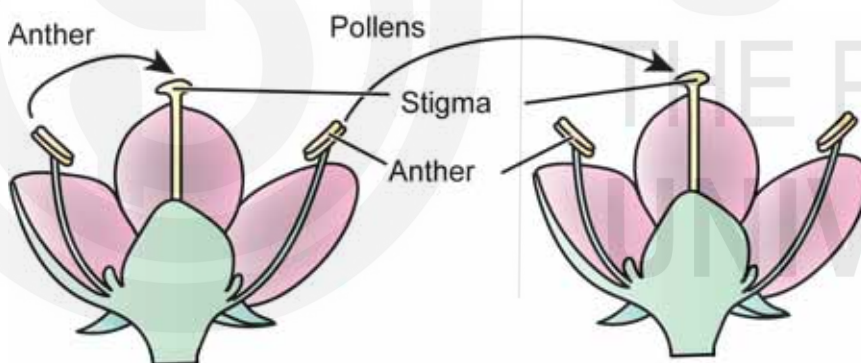


Fig. 10.1: Self-pollination in plants.

10.2.3 Advantages and Disadvantages of Self-Pollination

The structural and functional modifications of a flower in most species prevent or minimise the possibility of self-pollination. The main advantage of self-pollination is its certainty. However, self-pollination results in weaker progeny on account of **inbreeding depression**. In many species, self-pollination does not result in fertilization. This is because the pollen germination or growth of pollen tubes in the stigma or style is inhibited. For effective fertilization to take place, pollen has to come from another plant. This is genetically controlled and considered as a primitive character.

Self fertilization can be prevented in cases where flowers are not perfect i.e., lack any of the essential reproductive structures i.e. stamens or carpels in either dioecious or monoecious plant. When the male and female flowers are borne on different plants the condition is called **dioecious**. Self fertilization is

also prevented in flowers where stamens and petals mature at different times. In some species, anthers release pollen while stigma tissues are still immature.

Self- pollination leads to self fertilization. It shows advantages such as greater reliability especially in species in which individual is uncommon or separated by long distances. The parental characters are preserved. There is no wastage of pollen and it can occur in any flower i.e. they need not to be showy and colourful. The major limitation of this type of pollination is that it is a type of inbreeding and result in loss of vigour in offsprings.

SAQ1

- a) Choose the correct option from the following :
- i) The conditions in which male and female flowers are present on the same plant.
 - a) monoecious
 - b) dioecious
 - c) protoandry
 - d) dichogamy
 - ii) The condition in which the stamens mature earlier than the pistils.
 - a) dichogamous
 - b) dioecious
 - c) protoandrous
 - d) monoecious
 - iii) In which flowers, the position of anthers and stigma prevent self - pollination to occur.
 - a) herkogamous
 - b) dichogamous
 - c) protoandrous
 - d) monoecious
 - iv) The condition in which pollination occurs in a closed flower
 - a) chasmogamous
 - b) cleistogamous
 - c) dichogamous
 - d) herkogamous
 - v) The type of pollination in which pollen grains are transferred to the stigma of the same flower.
 - a) geitonogamy
 - b) homogamy
 - c) autogamy
 - d) cleistogamy
- b) Define the following terms:
- i) Pollination
 - ii) Geitonogamy
 - iii) Chasmogamous flowers
 - iv) Dioecious
- c) Explain the statement cross-pollination is advantageous to plants.

d) Answer in one word:

- i) The condition in which androecium and the gynoecium do not mature at the same time.
- ii) The condition in which pistils mature first then stamens.
- iii) The difference in the length of the style and stamens prevent self-pollination.
- iv) The condition when the male and female flowers are borne on different plants.

10.2.4 Cross-Pollination

You know that cross-pollination is a process of transfer of pollen from the anther of a flower from one individual to the stigma of another plant individual of the same species (Fig. 10.2). This process also termed xenogamy, is mediated by several physical and biological agents and brings the pollen from other plant individuals hence results in the production of offsprings which are genetically diverse and different from either parent. The genetic heterogeneity proves advantageous for plants. The offsprings are vigorous and better adapted to adverse environmental conditions; hence cross -pollination shows a wider distribution among angiosperms which we are going to discuss later. The cross pollination offers various benefits, hence the flowering plants (bisexual) have evolved several ways of preventing self- pollination. These include self - incompatibility, dichogamy herkogamy and heterostyly. These adaptations prevent self-pollination and induce cross pollination the common ones are discussed below:

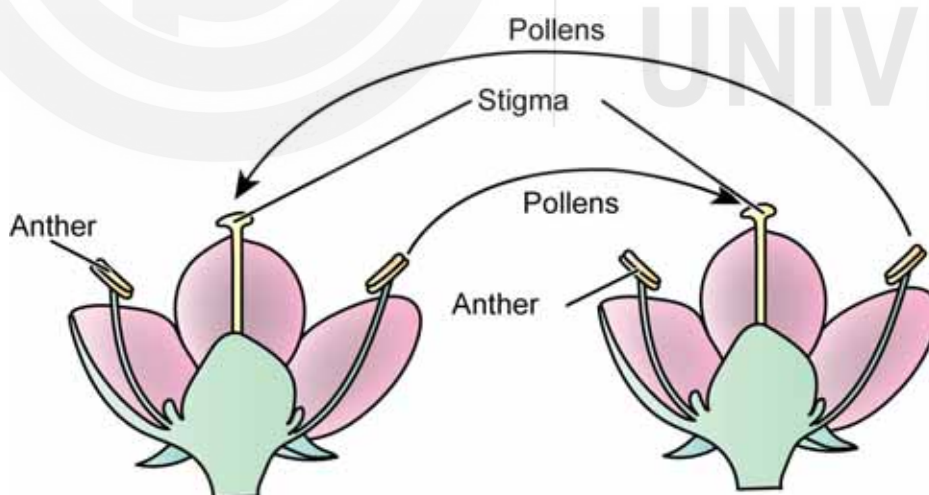


Fig. 10.2: Cross-pollination in plants.

- a) **Self- incompatibility** - it is a condition in which the pollens grains of a flower do not germinate at all or grow very slowly if carried on to the stigma of the same flower. There is an inhibition of the pollen growth on the stigma or in the style. In *Malva*, the pollen do not grow on the stigma of the same flower, and in *Petunia* incompatibility reaction occurs in the style.

- b) **Dichogamy** - In some flowers the condition is found in which the stigma becomes receptive before the pollen of the same flower matures or the anthers shed their pollen before the stigma of the same flower become receptive. The condition in which androecium and the gynoecium do not matures, at the same time is called **dichogamy**. In these species, the dehiscence of anthers and receptivity of stigma of a flower do not coincide. In sunflower, the anther dehisces before the stigma of the flower becomes receptive and thus self-pollination does not occur. The condition is called **protoandry**. In **protoandrous** flowers, the stamens mature earlier than the pistils. In contrast in **protogynous** flowers, the pistil matures first and the stamens mature later, for example *Mirabilis*.
- c) **Herkogamy** - In some plants, the position of anthers and stigma rule out self- pollination. The structural adaptations prevent pollen grains from coming in contact with the stigma of the same flower. In most of **herkogamous** plants, stigma projects beyond the level of anthers and as a result the pollen of the same flower cannot land on the stigma.
- d) **Heteromorphy** - Some plants bear morphologically distinct or dissimilar flowers. The heteromorphic flowers bearing two kinds of stamens and styles are referred as dimorphic flowers e.g., primrose (*Primula vulgaris*). In this plant two types of flowers are present. Some are long styled or pin eyed. These flowers bear carpel with long style, stigma with large papillae but short stamens and small pollen. The other type of flowers are short styled or thrum eyed, these flowers bear carpels that have short styles, small stigmatic papillae but long stamens and large size of pollen. The difference in the length of style and stamens and size of pollen and stigmatic papillae prevent self-pollination. The large pollen grains of thrum flower get better captured on long papillae of pin flower and small pollen of pin eyed flowers get caught in stigma with smaller papillae of thrum flowers.

In some species cross-pollination is achieved because of **heterostyly** (Fig.10.3). In this condition, plants have flowers with long pistils and short stamens, or long stamens and short pistils e.g., *Primula*. In this conditions, both long styled flowers i.e. which possess a long style present much above the anthers, or short styled flowers i.e. having stigma which is half the length of the corolla tube present below the anthers have been found.

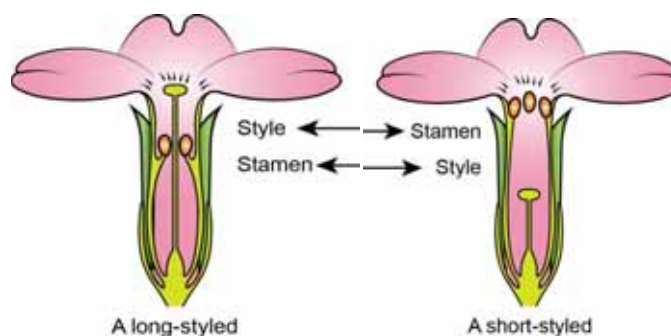


Fig. 10.3: Flowers showing heterostyly.

In members of Caryophyllaceae, the style is much longer than the stamens. Due to this the pollen of the flower fails to reach the stigma. In

members of the family Orchidaceae, the pollens of the anther sac are united to form a single compact unit called pollinium, hence the pollen are restricted from reaching the stigma of the same flowers

- e) **Diecliny** - This is a condition in which the male and female reproductive parts of flowers in plants i.e. anthers and stamens are present on different flowers. The flowers are unisexual and as a result they will not be able to self pollinate

10.2.5 Advantages and Disadvantages of Cross Pollination

Cross pollinated plants have many advantages as compared to self-pollinated plants. Cross-pollination increases the reshuffling of genotypes which improves the opportunity of new genetic combinations. This is because the sperm and egg cell from different plants unite resulting in a new combination of genes

- The plants are better adapted than parents. This is because of increased fitness caused due to genes inherited from the other individual's of same species. Variations in the gene pool prepare populations to counter stress conditions and changes in the environment.
- The plants produced through cross pollination are favorable for evolution.

Cross pollination also have some drawbacks. The major limitation is that the process is not certain, as it is dependent on favourable conditions and presence of pollinators. The process also involves expenditure of resources as plants need to produce more number of pollen grains as compared to self pollinated plants. Moreover flowers need to spend resources to produce large colourful flowers, to attract pollinators. The pollination is not always certain because a pollinating agent is required. In animal pollinated species, nectar and pollen need to be present in surplus to facilitate pollination

10.3 POLLINATION MECHANISMS

Pollen grains are transported to the stigma by a variety of biotic and abiotic mechanisms. Abiotic means mainly include water and air, while the biotic means include insects, birds, animals, bats etc. We will discuss here some of the common agents that mediate cross-pollination.

10.3.1 Anemophily

It is commonly known as wind pollination. It occurs in plants which possess small, unisexual flowers having short, non-showy perianth. The flowers in these plants lack odour and nectar, hence unattractive to insects and other living vectors. Pollens are produced in large quantities and pollen grains are light, dry with smooth walls. Pollens are released on warm and dry days when wind speed is high. Anthers and style tend to be erect and long to facilitate pollen dispersal. In most of the flowers the stigma of the pistil increases in size and becomes feathery to capture and hold the flying pollen. Such type of pollination has been known in members of Fagaceae (oaks), Betulaceae (birches), Salicaceae (poplars and willows) and many of the Poaceae (grasses) members.

In plants showing anaemophily, staminate, (male) and pistillate (female) unisexual flowers are produced in large number at the same time period. These flowers tend to aggregate in clusters to form catkins (Fig. 10.4).

The staminate unisexual flowers are elevated on slender stalks above the vegetative parts of the plants. In maize, the wind picks up pollen from the anthers and the rather large, heavy pollen grains fall on the corn tassels. The sticky corn silk on the young ears catches a lot of that pollen. The pollen grain germinates, produces a pollen tube that grows down the silk strand, and fertilizes an ovule in the ovary. In willows (*Salix* sp.), the unisexual staminate flowers are present in catkins that and are loosely arranged on the axis and shake open with the flow of wind. Some bisexual flowers may be present as vestiges or reduced sterile structures, reminding that unisexual flowers have evolved from bisexual flowers.

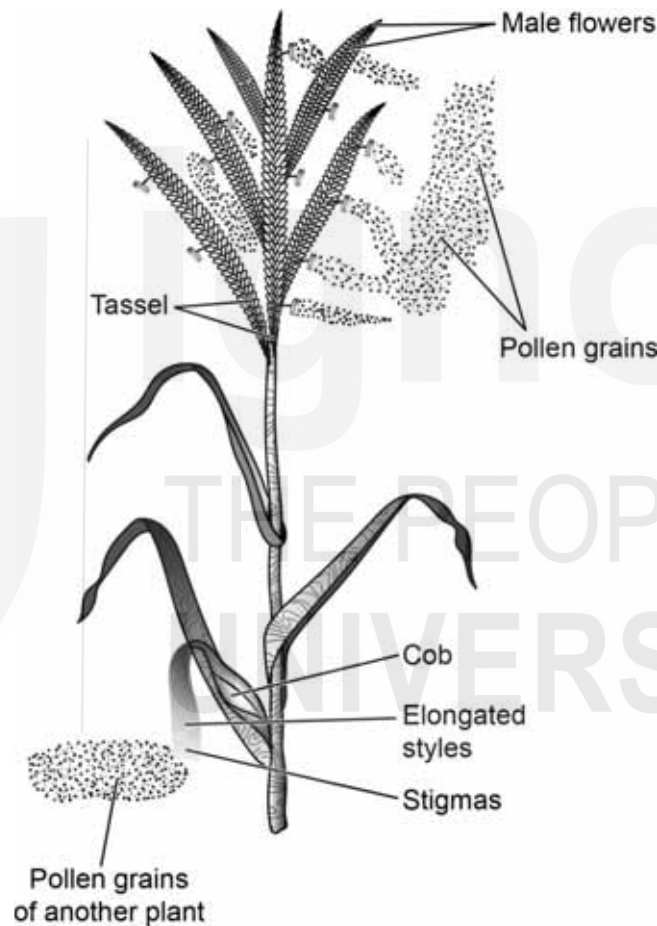


Fig. 10.4: Wind pollination in maize.

10.3.2 Hydrophily

In *Vallisneria*, an aquatic plant complete male flower is transported to the female flowers.

This type of pollination occurs with the help of water. It is also referred as water pollination. It occurs in aquatic plants having flowers at the surface of water or under the water. In aquatic conditions, a close correlation between the position of flowers and specific gravity of pollen is necessary. If female flowers are borne on submerged part, the pollen grains should possess a specific gravity equal to or slightly greater than water. If the female flowers float on the surface of water, than the specific gravity of pollen should be less than that of water so that pollen grains do not sink. The changes in specific gravity are regulated by the presence of starch grains.

The flowers are usually small and inconspicuous. These flowers are referred as hydrophilous flowers. These flowers are also categorised as **hyphydrophilous** and **ephydrophilous**. Hyphydrophilous flowers get pollinated when submerged in water while the ephydrophilous flowers get pollinated when floating on the surface of water.

In plants such as *Ceratophyllum*, 12-16 stamens are present in a flower and in each anther shows the presence of aerenchyma that helps it to float on the surface of water. The stamens get separated and float on the surface of water and the pollen gets liberated. The specific gravity of long pollen grains is same as that of water and hence they get freely distributed throughout the water. The pollens get trapped by the sticky lower surface of the style. This is a very common example of hyphydrophily.

In *Vallisneria* (Hydrocharitaceae), male and female flowers are produced under water. The female flowers are attached to thin, spirally coiled long, slender stalks. On maturity, tiny male flowers get detached from the stalk and float on the surface of water to assist in pollination (Fig. 10.5).

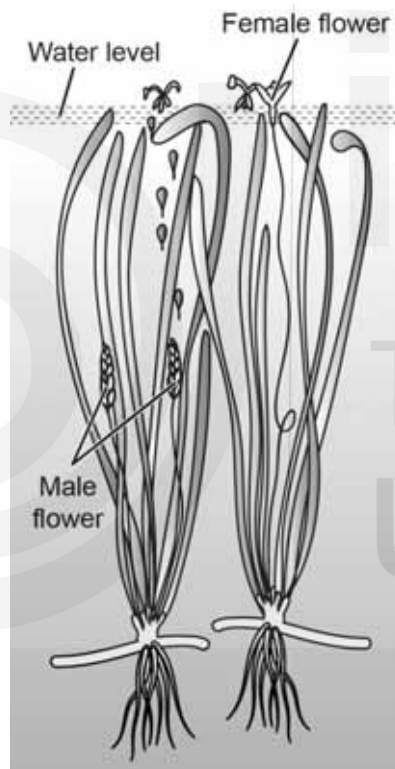


Fig. 10.5: Diagrammatic representation of pollination in *Vallisneria*.

The female flowers are raised to the surface by means of the long spiral stalk. The floating male flowers get lodged in the cup shaped depression formed around female flowers bringing anther in contact with the stigma. The pollen reaches the large stigma of female flowers. This plant represents an example of **ephydrophily**.

Sea grasses such as *Phyllospadix* present in the ocean waters possess elongated filiform pollen grains which are efficiently captured by the style and stigma of female flower. Another aquatic plant, *Zostera marina* produces long needle-like pollen and elongated pollen tubes. They float on the surface of water, come in contact with stigma and coil around it to complete the process of pollination.

10.3.3 Entomophily

This is also called as insect pollination. It is the most common type of pollination found in angiosperms. Various insects such as butterflies, beetles, moths, ants, bees and flies help in the transfer of pollen. Insect pollinators can be divided into three main groups:

1. Exclusive pollen seekers- (carpenter bees)
2. Exclusive nectar seekers- (moths and butterflies)
3. Pollen and nectar seekers- (honeybees)

Insect get attracted to flowers because of their specific colour, symmetry or scent. Some insects come to flowers to collect nectar which is present in the corolla tube or spur. Some flowers (*Cassia* sp.) lack nectar, while in others (members of Apiaceae, Euphorbiaceae) nectar is the main reward for insects. In some flowers nectar is freely exposed and easily available to short-tongued insects such as flies and beetles, while in others it is hidden by scales (*Ranunculus*) or covered by hairs or a short perianth tube (*Ricinus* and *Thevelia*). In some other flowers, the nectar lies completely concealed and nectar is taken with the help of long tongues. The concealed nectar means that it is enclosed in long corolla tube or spur. Insects also visit flowers to eat pollen which is one the source of food for them.

In insect pollinated flowers, the position of anther and stigma is such that when an insect takes food from the flower, it becomes dusted with pollen grains. It then transfers these pollen grains to the stigma of another flower during subsequent visit. Insects do not recognise the colors in the same way as they are by humans. Insects can see in the ultra violet range of electromagnetic spectrum (invisible to the human eye). Red colour appears black to them. Hence the insect's pollinated flowers have mostly yellow or blue colour and not red colour.

In case of bee pollination, the flowers are showy, colourful and fragrant (Fig.10.6). The specialised color patterns called **nectar guides** function to attract bees so that maximum number of pollens grains can be transferred. Bee pollination is referred as **hymenopterophily or melittophily**. The bees can also detect and distinguish between different odours, colours and sugar concentrations.

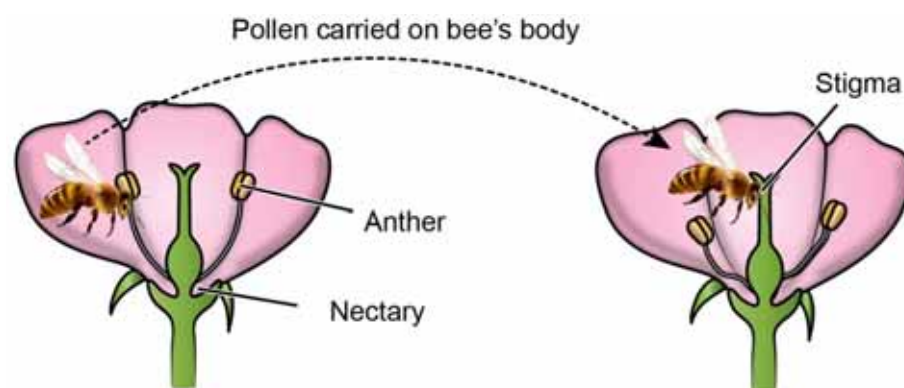


Fig. 10.6: Pollination by bees.

Bees perceive flowers which reflect yellow-green, blue-violet, and ultraviolet (UV) light. The petals in most of these flowers show the presence of colored lines or dark marks (dots, crosses, checks) which guide bees to the nectaries. The floral architecture ensures that insect transfers the pollen to the stigma. In garden nasturtium nectar is held in a long narrow tube or spur. The bee directs its proboscis into the tube and rubs it against the stigma on the way thereby releasing the pollen.

BOX: 10.1 Waggle Dance of Bees.

A honeybee locates a source of nectar or pollen and then she conveys that finding to other individuals by means of 'waggle dance'. This dance conveys a lot of information about the food source to the other bees. When the food source is near the hive, the scout performs a round dance. The dance involves series of circles on the comb alternating between clockwise and anticlockwise directions after every one or two circles. The nearby bees follow the manoeuvres of the forager now and then. The dancing bee gurgitates drop of nectar from its stomach, offering it to the nearby bees. The bees know about the presence of food from lingering odour of the flowers still adhering to the dancer's body and odour of the food regurgitated by the dancer. This information enables the bees to leave the hive in search of food of the correct odour in its vicinity. The rate of the dance conveys to the approximate distance of the food source from the hive. Sun indicates the direction of the food source. A dance at an angle of $60^\circ/120^\circ$ to the left/right signifies that the bees should follow a direction that forms the same angle (60° or 120°) with the line connecting the hive and the Sun. If food is located towards the Sun, the wagging portion is straight up on the comb. When the food is 20° to the right of the Sun, then wriggling will be 20° to the right of the straight up. A scout honeybee indicates the distance and direction of a newly found rich food source. She signals by the presence of food by means of a figure-of-eight dance to her hive mates. When the bee moves through the middle of the 8 she gives a number of tail wags, quickly when the food source is nearby and more slowly when it is farther away. For example, for a food source a mile off, she gives four wags.

Salvia has been studied as one of the common example of insect pollination. *Salvia* species exhibits a specialised 'turnapipe' floral mechanism which is suitable for bee pollination. In *Salvia*, the corolla is bilipped and the stamens are attached to the corolla tube, the stamens are separated by a long connective and the filament is attached to the connective in such a way that a lever mechanism is formed and connective can swing on the filament. Half of the anther is fertile and other half is sterile. The sterile portion joins together to form the sterile plate placed above the lower lip of the corolla (Fig. 10.7).

When bee/insect visits the flower for nectar its head pushes the sterile plate as a result of which the upper fertile lobe of the corolla lobe hits the insect on its back. Thus, pollen gets dusted on the back of the insects. When the bee visits another flower, the forked stigmas pick up the pollen from the back of the insect.

The bees collect pollen and sugar- rich nectar which further gets converted to honey for feeding the young. Bees have been considered as efficient pollinators because they visit several flowers until all the nectar is used up. Honey bees possess pollen basket. Their bristly hair attach pollen grains. They brush this pollen with their legs into the basket or on either sides of their

body. They return to hive after the baskets are tightly packed with pollen. Some of the pollen is dusted to other flowers visited resulting in pollination.

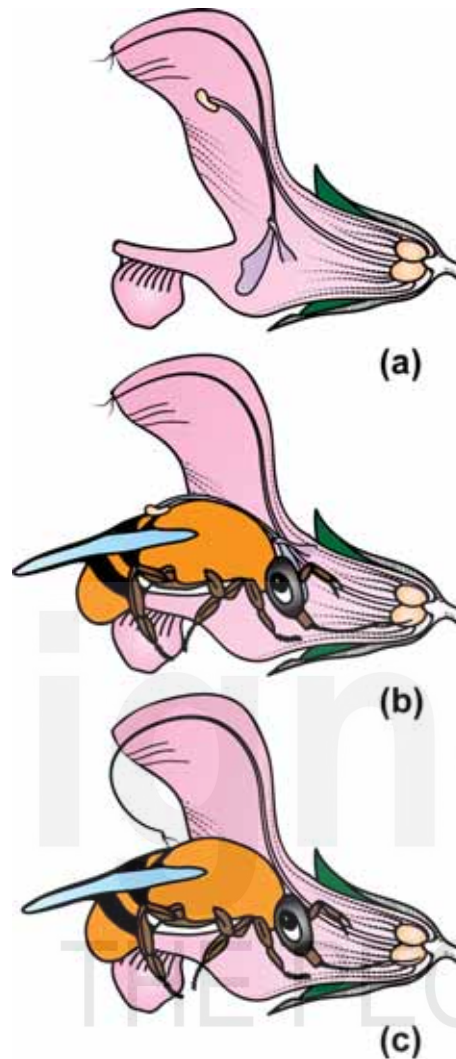


Fig.10.7: Pollination in Salvia. a) Flower of Salvia with a) bilipped corolla and stamens attached to corolla tube; b) sterile plate in the stamen being pushed by the bee; c) insect back being dusted with pollen from the anther.

Moths pollinate white, fragrant flowers. The pollination is known as **Phalaenophily**. The flowers do not possess nectar guides but have long nectar filled tubes or spurs. The nectar present at the base of long petal tube is available to moths which have long tubular mouth parts. Example- *Yucca* is pollinated by moths *Parategeticula* and *Tageticula* sp. The female moths lay their eggs within the ovary of the flower. These moths are obligately dependent on the *Yucca* species for completing their life cycle. Another example is a tobacco flower which is pollinated with *Carolina sphinx* moth. Species of *Cestrum* and *Nyctanthes* produce a strong fragrance at night to attract the moths.

The pollination that takes place with the help of beetles is called **Cantharophily**. It takes place in open flowers having fruity or foul odour. Some of these flowers also produce heat internally which helps in the dispersal of odour.

The pollination carried out with the help of butterflies is referred as **Psychophily**. Butterflies help in the pollination of flowers that produce colorful, showy fragrant flowers with nectar. Butterflies are active during the day and

visit a variety of flowers. Butterfly lands on the flower and uses its palpi and antennae as sensory probes. The flowers have long nectar filled tubes. The nectaries are usually concealed in the flower; hence the mouthparts of butterflies have become suitably modified to reach the nectary. The butterflies visit the attractive flowers and long coiled tube- like tongue of butterfly is adapted to suck nectar. It punctures the nectary to suck the nectar. In the process, the pollen is dusted on the tongue and head of butterfly.

In many of the orchids the pollination is facilitated by a wasp species e.g., *Ophrys speculum*, pollination is assisted by wasp, *Colpa amaea*. The smell and colour of the female wasp matches with that of orchid flowers. The male wasp mistakes the flower as it is a female partner and carries out pseudo-copulation. In the process the transfer of pollen takes place.

Pollination is carried out by flies is referred as **Sapromyophily**. The flies visit the plants in which the flowers possess foul odours or fetid odour that is like the smell of rotting flesh or decomposing organic matter. The flowers visited by flies are generally brown, white or yellow in color e.g., *Arum* and *Stapelia*. In *Arum* (Araceae), the odour and colour of the bracteate flower cluster (spadix) attract flies. The visitor fly slide down the spadix, trapped in the covering of bracts and hence carries the mature pollen. Inside the flower, flies are unable to escape until the hairs lining the funnel wither away by which time the stamens would have shed their pollen.

In many plants, the pollination is carried out by Bumblebees. These *Bombus* spp. foragers have shown potential to pollinate selected flower species, particularly in high altitude areas. These bees visit different plant species and concentrate on the most remunerative species. Pollination occurs through a method called buzz pollination in which rapid vibrating motion of the bee result in release of large quantities of pollen onto the bumble bee. Like honey bees, the bumble bees collect pollen for their young, and nectar for store in their nest. Temperature and other physical factors greatly affect the foraging activity of bumblebees.

Pollination by insects such as honey bees, bumble bees, is very important as more than 70% of the economically important plants are pollinated by them.

Box 10.2: Pollination in Figs

In wild fig (*Ficus carica*) varieties such as Caprifigs, the pollination is done by wasps (*Blastophaga glossurum*). The inflorescence in figs is hypanthodium which consist of hollow flask shaped and succulent receptacle enclosing a cavity which opens outside by a pore. The inner surface of receptacle bears sessile and unisexual flowers. The male flowers are present at upper end while the female flowers are present at the base. The female flowers are either long styled or short styled called gall flower. The wasp lays its eggs in the ovaries of gall flowers. The female wasp enters the hypanthodium through apical pore reaches the ovaries of gall flowers, lays its eggs and while moving out gets dusted with the pollen from the anthers of male flowers. The wasp having pollen when it goes to another hypanthodium enters the long styled flowers depositing the pollen on the surface of stigma causing pollination.

10.3.4 Ornithophily

This is also called as bird pollination. This type of pollination occurs in plants that produce red, yellow or orange flowers. The flowers are large tubular, cup-shaped or urn shaped and secrete nectar. The tube is formed from the tightly wrapped perianth parts. The flowers pollinated by birds are usually bisexual i.e., possess both stamens (male) and pistils. They usually have an inferior ovary which protects the ovules. The stamens brush against the head or breast of the visiting bird. The bird-pollinated flowers are comparatively bigger in size and open during the daytime. The flowers can be sturdy firmly attached to the stem or swinging which generally attract hovering birds. The flowers are generally bilipped (bilabiate), tubular, and having protruding stamen arrangement and nectar is contained in spurs. The flowers possess abundant nectar but lack smell. The birds that help in pollination include humming birds, sun birds, honey eaters. Most birds have a good colour sense and excellent vision but have poor sense of smell. The birds feed on small insect attracted by the nectarines. Some of the Ornithophilous species include *Fuchsia*, *Hibiscus* and many species belonging to the families Orchidaceae, Fabaceae (Leguminosae) and Cactaceae. *Bombax ceiba* (semal) and *Butea monosperma* (dak) are common bird-pollinated trees in India. Most bird-pollinated trees flower in February-March period, which is also the breeding season of birds when they require maximum food resources.

The Indian coral tree (*Eiythrina*) is pollinated by parakeets and woodpeckers. The flowers bear tubular corolla with abundant sugary nectar. Species of *Bignonia* are pollinated by humming birds. Humming birds are strongly attracted by red or orange colored flowers (Fig. 10.8). The humming birds extract nectar while hovering over the flower when they visit pendent flowers for pollination. Sun birds perch on a branch and suck out nectar from the erect flowers.



Fig.10.8: Pollination by humming bird.

10.3.5 Cheiropterophily

Pollination brought by bats is called Cheiropterophily. It occurs in those flowers that open at night (nocturnal anthesis). The flowers are large, white dull red or yellow and produce abundant pollen and nectar secreted by hypanthium or perianth tube. The flowers produce strong unpleasant smell usually like fermented fruit. Bats get attracted to the flowers by the scent and lap up the nectar. The movement to different flowers, transfers the pollen. The flowers in

these plants are borne singly or in clusters away from branches, either projecting above the tree canopy as in Indian trumpet flower, *Oroxylum indicum*, or hanging below as in African sausage tree (*Kigelia pinnata*) and baobab tree, (*Adonsonia digitata*). Bat pollinated flowers are bell shaped, or open saucer shaped with many stamens or suspended with stamens bunched in a protruding brush. A bat clasps the flower with its claws and during nectar lapping its back gets dusted with pollen.

10.3.6 Zoophily

Mammals feed on the nectar of several plant species. These pollinators mainly include marsupials, primates, and rodents. African lily, *Massonia depressa* is pollinated by rodent species from the South African region. In animal pollinating species, nectar and pollen need to be present in surplus to facilitate pollination. Animals pollinate flower species with characteristic morphology, colour, and odour. Red flowers having little odour are generally pollinated through animals. Animal pollinators get benefitted by getting nectar. Flowers of *Aspidistra lurida*, *Chrysanthemum leucanthemum* and some Araceae are pollinated by snails and slugs. These flowers are described as **Malacophilous**. Animals visit the flowers for pollen and nectar.

Nectar : Nectar in flowers is secreted by nectaries or glands present on thalamus, perianth or ovarian disc. It contains sugars (mainly glucose, sucrose and fructose), water and other substances such as proteins; amino acids, lipids, alkaloids, phenolics, antioxidants, saponins, dextrans, organic acids and organic compounds in small amounts. Their sucrose content can range from 5% to 80%. Nectar volume influences the distance travelled by pollinators and also number of flowers visited per plant. Competition for nectar seems to be an important determinant of the extent to which various species of bees utilise the flowers of *Agave schottii*.

Nectaries can be of two types- floral or extrafloral which is outside the flower in the inflorescence, petiole or leaves and attract insect visitors. Their function is often to distract the non-pollinating insects such as ants and preventing them from coming to flowers. These nectaries usually secrete nectar continuously. The activity of floral nectaries is periodical and correlated with insect visits. Floral nectaries are usually concealed well within the flower. The time and amount of nectar secretion is often correlated with the time of the pollinator activity. In plants which flower at night, most of the nectar secretion occurs in the evening and declines after midnight. In butterfly-pollinated plants, flowering occurs during the rainy season, usually in the months of July-September and nectar production is also very high at this point of time.

Pollen : Pollen stores starch, lipid and proteins and is nutritious for beetles, flies, bees and bats. Pollen consumption (known as pollinivory) is considered one of the precursor to pollination carried by vectors. In a mutualistic process it has been seen that pollen-feeders deliver unconsumed pollen to the female reproductive organs of their host plant.

Heat : The thermogenic flowers maintain a constant high internal temperature irrespective of temperature fluctuations in the outside environment. These

flowers are generally large and possess high surface to volume ratio. These flowers occur in plant families such as Araceae, Aristolochiaceae, Nelumbonaceae, Annonaceae, Arecaceae and Cyclanthaceae. The Amazon water lily, *Victoria amazonica* combines heat production with a change in petal colour to control the behaviour of beetle pollinator. The flowers of this plant are about 20 cm wide and have beautiful white petals on the first day of anthesis. At this time the flower temperature rises to about 10°C above the ambient air and emits a strong fruity odour. The floral display and the scent attract a large number of beetle pollinators. As the evening approaches, the petals gradually close, trapping the insects inside. The petals reopen the next day afternoon and allow the beetles to crawl out through the stamens which dust them with pollen. Insects do not get attracted to the second day flower because it is not strongly scented but instead fly to first-day flowers, cross pollinate them and repeat the cycle. Aquatic plant lotus, *Nelumbo nucifera* is another example of such flowers. It also adopts the same strategy for pollination. Many beetles are endothermic and require high temperatures in their thoracic muscles for activity such as flight.

SAQ 2

- a) Choose the correct option :
- i) Hymenopterophily is the term given for pollination by
 - a) birds
 - b) bats
 - c) Bee
 - d) insects
 - ii) Pollination brought by bats is called
 - a) cheiropterophily
 - b) sapromyophily
 - c) cantharophily
 - d) phalaenophily
 - iii) The flowers that maintain a constant high internal temperature irrespective of temperature fluctuations in the outside environment.
 - a) cantharophilous
 - b) sapromyophilous
 - c) thermogenic
 - d) phalenophilous
 - iv) The pollination of flowers with the help of snails is termed as
 - a) cheiropterophily
 - b) sapromyophily
 - c) malacophily
 - d) phalaenophily
 - v) In wild fig (*Ficus carica*) varieties, pollination is carried out by
 - a) bees
 - b) snails
 - c) butterflies
 - d) wasps
- b) Describe the major characteristics of the insect pollinated species.

10.4 POLLEN-STIGMA INTERACTION

Pollination transfers the pollen to the stigma. The pollen after reaching the stigma germinates and the pollen tube carries the sperm/male gamete to the embryo sac present in the ovary so that double fertilization takes place. Fertilization process involves complex events with highly regulated cell-cell interactions and signaling between the pollen and the pistil. The success depends on series of events such as delivery of pollen to a stigma, pollen hydration, germination, directional growth through the pistil to the ovary, finally and fertilization.

In a compatible pollination, pollen grains reach the stigmatic surface and undergo-adhesion, hydration and germination to the stigmatic cells leading to formation of pollen tubes. The actively growing pollen tubes are often capable of penetrating the stigmatic cell walls to grow down through the style and finally reach the ovary to deliver sperm for fertilization. In self-incompatible pollination, these steps may be blocked. Understanding of pollen to stigma compatibility and self- incompatibility is required for the study off fertilization and breeding in plants. The mechanism of pollen-stigma interaction determines compatibility or self- incompatibility.

A number of barriers are present in flowering plants that prevent pollen from germinating or pollen tube to elongate in incompatible pollination. These barriers also rule out germination of foreign (other species) pollen that may land on stigma. The barriers act immediately after pollination, and prevent pollen germination; the chemical reactions between pollen and carpels prevent pollen growth. In incompatible systems, as the pollen tube grows, the stigma and the style proteins present on the pollen tube surface act as a barrier. The self-pollination in plants is inhibited because of compatibility barriers.

If the protein is produced by the gene in pollen grain that matches the incompatibility gene in the carpel, the stigma and the style block further growth of the pollen tube. In self-pollination, all pollen tube genes match those of the stigma, style and blocking of pollen tube growth occurs. In some cases, the proteins deposited on the outer surface of the developing pollen grain are produced by the anther tapetum. The proteins produced by the incompatibility genes block germination of the pollen grain. The interaction between the pollen tube and style is regulated by a haploid genome (pollen tube) and a diploid genome (carpel) or both the diploid genomes (anther and carpel). The diploid interaction involves greater pollen rejection.

10.4.1 Wet and Dry Stigma

It is the specialized part of the pistil which receives pollen grains during pollination. The pollen received by stigma germinates and produce pollen tube (Fig 10.8). The nature of the stigma show considerable variation. Based on their nature, they have been classified into two types: dry and wet stigma. The differentiation is based on the presence or absence of stigmatic exudates at the time of pollination.

Wet stigmas- This stigma is covered with surface cells that often lyse to release secretions such as proteins, lipids, polysaccharides, and pigments.

This type is found in plants like *Aegle marmelos* and *Petunia hybrida*. The secretion is primarily lipidic or aqueous and carbohydrate-rich.

Dry stigmas- This stigma has intact surface cells and are covered by a primary cell wall, a waxy cuticle, and a proteinaceous pellicle. The cells protrude as papillae. This type of stigma has been reported in cotton. In dry stigma, where there is no stigmatic secretion, initial adhesion of pollen to stigma depends on the pollen wall. Tryphine, a lipid-rich pollen coating, is found to be important in adhesion to the dry stigma.

The exudates in wet stigma are secreted by endoplasmic reticulum or stigmatic papillae. The exudates are highly viscous, refractive and adhesive. The exudates contain lipids, proteins, glycoproteins, carbohydrates (glucose, fructose, and sucrose), amino acids and phenols. The lipids protect stigma from desiccation and regulates the availability of water to the pollen. The phenolic compounds protect stigma from insects and other pests. The enzymes diffusing out of pollen grains on the stigma release free sugars from phenolic glycosides which provide proper osmotic conditions for germination (Fig. 10.9) and tube formation.

In developing stigma, epidermis is covered by thin cuticle and densely cytoplasmic sub epidermal cells are present. These cells elongate to form secretory zone with large schizogenous cavities filled with lipid secretion. The cells of the stigma contain numerous amyloplast and large amount of lipid globules which coalesce and migrate to the peripheral part of the cytoplasm and eventually out of the cell. The lipid exudates accumulate between the cell wall and the cuticle. In the secretory zone, the exudates fill large intercellular schizogenous cavities. At the time of anthesis, the epidermis becomes disorganised, cuticle is discarded and the accumulated exudate spreads over the entire surface of stigma.



Fig 10.9: Pollen germination on stigma.

Dry stigma is covered with long unicellular hairs. The stigmatic hairs show distinct and continuous cuticle which is closely pressed to the wall. The pellicle (extracellular proteins) is present on the stigma. The stigmatic surface also bears lipids and phenolic substances. Below it are present several layers of intercellular spaces. With time the spaces get occupied by pectin like substances.

In early stages of development, the epidermis of stigma is covered with a cuticle which generally has an extracellular proteinaceous lipoidal layer called pellicle. This layer helps in the capture and hydration of pollen grains and also serves as a recognition site during pollen pistil interaction. In wet stigma both epidermal and subjacent cell layers release exudates (Fig.10.10).

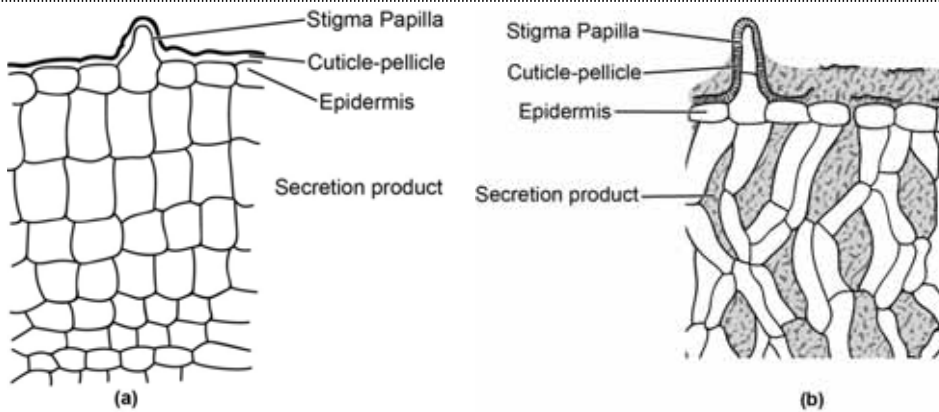


Fig.10.10: Diagram showing the presence of exudates on the stigma surface.
a) Presence of thin pellicle layer; b) Accumulation of exudates between the cuticle-pellicle layer and epidermal cells.

The exudates of the subepidermal cells accumulate in the intercellular spaces and form an interconnecting system, while the exudates from the epidermal cells accumulate below the cuticle-pellicle layer. The continuous secretion of the exudates in the intercellular spaces leads to enlargement of epidermal cells followed by loosening and this causes the transfer of exudates from the adjacent intercellular spaces to reach the subcuticular zone (Fig. 10.11).

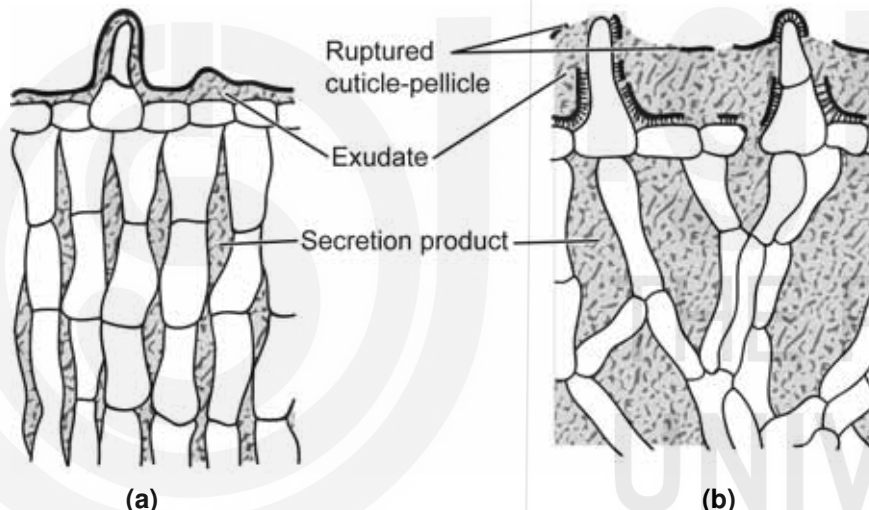


Fig.10.11: Diagram showing the presence of exudates on the stigma surface.
a) accumulation of exudates in intercellular spaces; b) mature stigma.

Finally the cuticle-pellicle layer is disrupted releasing the exudates. The exudates are secreted by the endoplasmic reticulum (ER) and extruded by exocytosis. In dry stigma the cuticle is discontinuous and the components of the papillae get out through these discontinuities (Fig. 10.12).

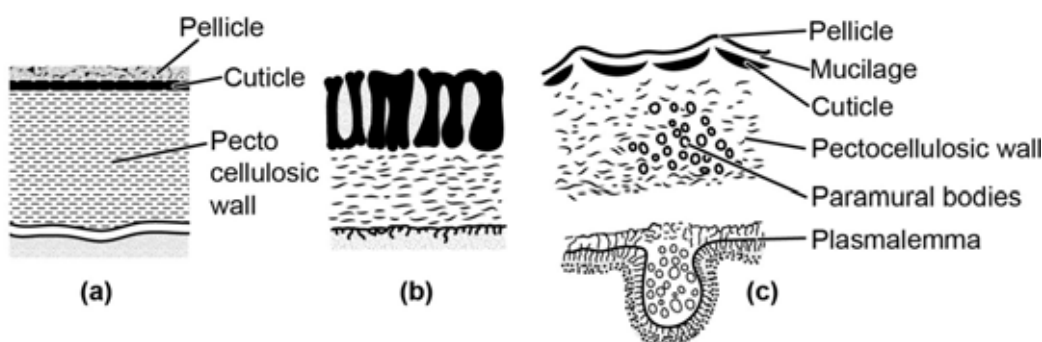


Fig. 10.12: Structure of wall of stigmatic papillae in dry stigma. a) structure of dry stigma having pellicle, cuticle and pectocellulosic wall; b) cuticle with cavities having secretion material as noted in *Crocus*; c) differentiation of pectocellulosic wall as noted in *Poaceae* (after Shivanna, 1979).

The pollen–stigma interface can differ from species to species as a result of the wide variability in the morphology and content of stigmatic exudates, and pollen wall. The pollen-stigma interface changes as pollination progresses, becomes considerably stronger over time, with different types of adhesive contacts supplementing each other (Fig.10.13).

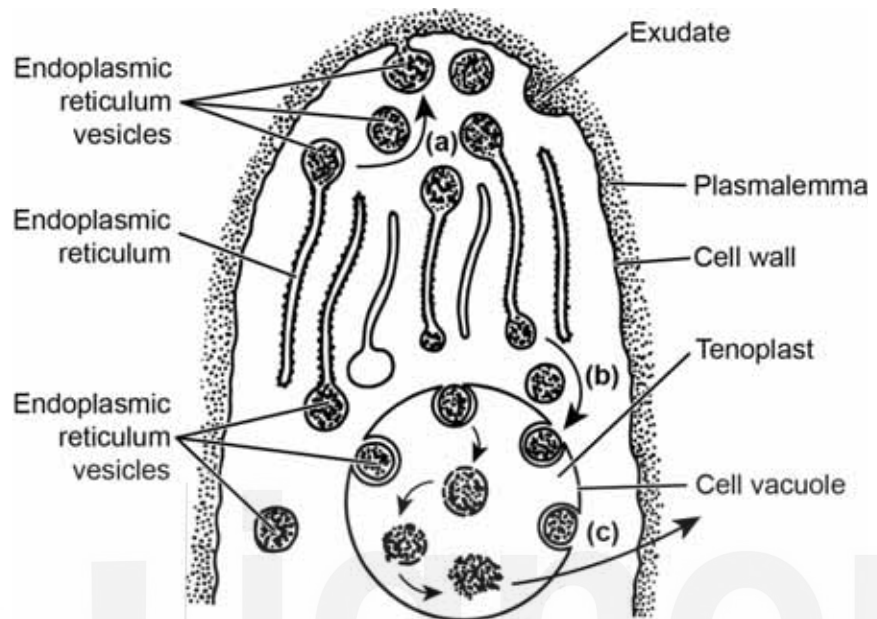


Fig.10.13: Proposed secretion pathways in *Aptenia cordifolia* a) Granulocrine secretion of exudates by exocytosis; b) Inclusion of vesicles into the vacuole, and the mixing of the vesicular contents with the vacuole substance following membrane dissolution; c) Degeneration of protoplast leads to holocrine excretion of mixed exudates.

The adhesion of pollen depends upon the pollen exine. Thus, pollen capture most likely depends on biophysical and/or chemical interactions between the stigma surface and the polymers of the pollen exine. The exine-mediated adhesion is followed by mobilization of the pollen coat leading to mixing of lipids and proteins to form a “foot” of contact on the stigma surface. The proteins and lipids in the pollen coat, and proteins on the stigma surface, contribute to adhesion, most likely at a later stage of contact. The lipid-rich stigma exudate of plants with wet stigmas is thought to be functionally analogous, to the pollen coat.

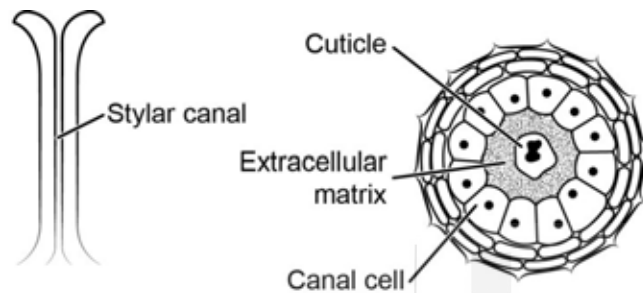
Besides having a major role in pollen-pistil interaction, the different components of the stigma secretion are also known to be involved in other functions such as :

- i) lipoidal component prevents evaporation and wetting by acting as a liquid cuticle;
- ii) phenolics and proteinase inhibitors provide protection against insects and pathogens; and
- iii) the stigmatic exudates serve as a nutrient source for pollinating insects.

10.4.2 Open and Closed Style

The style is a tubular structure that connects the stigma with the ovary and is mainly of two types, viz., solid (closed) and hollow (open)

Open style - In the hollow style, (Fig.10.14) the central core has one or more canals which normally correspond to the number of carpels. These are referred as styler canals. The styler canal is bordered by one or a few layers of glandular cells called canal cells. These canals are lined with glandular epidermis with a thick outer tangential wall. The wall of the canal is smooth but highly convoluted (*Aegle*, *Fritillaria*). In some plants such as *Citrus*, the inner tangential wall of canal cells is also thick and made up of fibrillar homogenous material. The canal cells are large and become multinucleate. The cytoplasm becomes rich in organelles such as mitochondria, dictyosomes, free ribosomes or polysomes, smooth and rough ER. The major portion of the secretion product is transported to the canal cells through plasmodesmata.



10.14: Diagrammatic representation of hollow style and longitudinal section and showing continuous stylar canal and stylar canal in TS.

In *Lilium* sp. dictyosomes secrete non-cellulosic amorphous polysaccharide containing mucilage at early stages. The secretions are retained with the help of thin and continuous layer of cuticle until pollination. The stigmatic papillae lack the secretory zone seen in canal cells and stigmatic exudates appear before pollination. The stigmatic exudate can thus be considered as a secretion product of canal cells transported through intercellular spaces. Pistils of *Lilium longifolium* secrete large quantities of exudate which accumulates on the surface of stigma in the form of droplets. The stylar canal is also filled with aqueous solution containing high molecular weight protein and polysaccharides –galactose, arabinose, rhamnose, glucuronic acid. The exudates also play a role in protecting the fragile pollen tubes during their growth. The stylar secretion in hollow style shows esterase and acid phosphatase activity.

Closed Style - The solid style (Fig.10.15) has a central strand of transmitting tissue composed of elongated cells. Transmitting tissue starts from the stigmatic secretory tissue and traverses the entire length of the style. The transmitting tissue consists of thin walled cells having plasmodesmata. The cells of the transmitting tissue have thin transverse walls but thick lateral walls. The different concentric layers are also present. The innermost wall layer is composed of pectic substances and hemicelluloses, surrounding this is wall layer 2 which is darker, thinner and similar to wall layer 1 in composition. Wall layer 3 is loosely textured, rich in pectin substances and contains small amount of cellulose. Wall layer 4 is represented by middle lamella region and is rich in pectin. The cells of the transmitting tissue contain mitochondria and active vesicles forming dictyosomes. The plastids are large with numerous amyloplasts, polysomes and abundant endoplasmic reticulum, vacuoles (spherical or ellipsoidal). The cells of the transmitting tissue do not divide but show elongation.

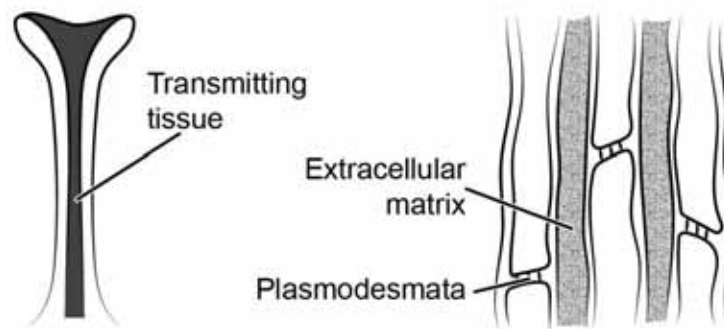


Fig.10.15: Diagrammatic representation of solid style with transmitting tissue in longitudinal section and showing plasmodesmata connection between lateral walls.

Intercellular substances of pectinaceous nature surround the cells of the transmitting tissue. It also contains proteins, glycoproteins, and often lipids. A number of transmitting tissue-specific proline-rich proteins have been localised in the intercellular matrix. The cells of the transmitting tissue generally possess numerous mitochondria, active dictyosomes, rough endoplasmic reticulum, plastids and ribosomes. Pollen tubes grow down the style through the intercellular matrix of the transmitting tissue.

SAQ 3

- a) Fill in the blanks:
 - i) In closed type of styles, a compact core of is present.
 - ii) The stylar canals are present in type of style.
 - iii) stigma is covered by sticky secretion.
 - iv) The nectaries which occur outside the flower in the inflorescence, petiole or leaves are called
 - v) The stigmatic exudates serve as a nutrient source for
 - b) What is transmitting tissue? Explain.
 - c) Open style is different from the closed style in terms of structure and production of exudates. Explain.
-

10.5 SUMMARY

- The transfer of pollen from the stamen to the stigma of the carpel before fertilization is termed as pollination.
- Two types of pollination have been recognized. Self-pollination involves the transfer of pollen from anther to stigma of the same flower (autogamy) or another flower on the same plant (geitonogamy), while cross pollination involves the transfer of pollen from one flower to the stigma of a flower from another plant of the same species (xenogamy).

- Bisexual flowers have some adaptations that prevent self- pollination. These include self sterility, dichogamy, herkogamy, and heterostyly. In these conditions, dehiscence of anthers and receptivity of stigma of a flower do not occur at the same time either stamens mature earlier than the pistils (protoandry) or pistil becomes receptive before anther dehiscence (protogamy). In some species length of the pistils and stamens varies or flowers are unisexual.
- Biotic and abiotic pollinators transfer pollen grains to stigma, which also possesses strong adhesive interactions to retain pollen grains. Pollination is facilitated by various agents such as wind (anemophily), water (hydrophily), large animals (zoophily), birds (ornithophily), insects (entomophily) or bats (cheiropetrophily).
- A number of barriers present in flowering plants prevent undesired pollination. The barriers act during the pollen-stigma interaction. The chemical reactions between pollen and carpels prevent pollen growth. In incompatible systems, as the pollen tube grows, the stigma and the style proteins interact with the pollen tube surface. If the protein is produced by the incompatibility genes germination of the pollen grain is blocked.
- The stigma has been classified into two types- wet and dry stigma depending upon the presence or absence of stigmatic exudate. Wet stigma is covered by sticky secretion but dry stigma lacks secretion. The style has been classified as open and closed types. In open type, the stylar canal lined with glandular and secretory cells is present. In closed type, a compact core of transmitting tissue is present.

10.6 TERMINAL QUESTIONS

1. Enlist the differences between
 - i) Self- pollination and cross pollination
 - ii) hydrophily and zoophily
 - iii) wet and dry stigma
 - iv) Psychophily and hymenophily
2. Describe the type of pollination noted in *Salvia*.
3. Exudates of the stigma are very useful to the pollen during germination. How?

10.7 ANSWERS

Self-Assessment Questions

1. a)
 - i) a) monoecious
 - ii) c) protoandrous
 - iii) a) herkogamous
 - iv) b) cleistogamous
 - v) c) autogamy

- b) i) Pollination - the transfer of pollen from dehiscing anthers to the pistil or the process of transfer of pollen from anther (male reproductive part) to the stigma (female reproductive part) of the same flower or different flower is called as pollination.
- ii) Geitonogamy - This type of pollination occurs between the two different flowers present on the same plant.
- iii) Chasmogamous flowers - the flowers in which pollination occurs in the open flowers.
- iv) Dioecious - the condition in which male and female flowers is borne on different plants.
- c) Cross-pollination increases the reshuffling of genotypes which improves the possibility of new genetic combinations. In this, sperm and egg cell from different plants unite resulting in a new combination of genes which might be better adapted than the parents. This leads to more fitness. The variations in the gene pool allow populations to fight changes or stresses present in the environment. Hence cross pollination is favourable for evolution.
- d) i) dichogamy
ii) protogynous
iii) heterostyly
iv) dioecious
2. a) i) c) bee
ii) a) cheiropterophily
iii) c) thermogenic
iv) c) malacophily
v) d) wasps
- b) Plants that are pollinated by insects
- produce flowers that are showy, colourful and fragrant.
 - show specialised colour patterns called nectar guides that function to attract and guide bees and other insects to nectar source so that maximum number of pollen grains can be transferred.
 - have corolla which is bilipped and the stamens are attached to the corolla tube.
 - have long nectar filled tubes or spurs. The nectaries are usually concealed in the flower.
3. a) i) transmitting tissue
ii) open
iii) wet

- iv) extrafloral
- v) pollinating insects
- b) The transmitting tissue consists of thin walled cells having plasmodesmata. The cells of the tissue have thin transverse walls but thick lateral walls. The different concentric layers are also present. The innermost wall layer is composed of pectic substances and hemicelluloses. Surrounding this is wall layer 2 which is darker, thinner and similar to wall layer 1 in composition. Wall layer 3 is loosely textured, rich in pectin substances and contains small amount of cellulose. Wall layer 4 is represented by middle lamella region and is rich in pectin. The cells of the transmitting tissue contain mitochondria and active vesicles forming dictyosomes. The plastids are large with numerous amyloplasts, polysomes and abundant ER, vacuoles (spherical or ellipsoidal). The cells of the transmitting tissue do not divide but show elongation.
- c) In open style, the styler canals are present. The styler canal becomes lined with glandular and secretory cells which become dome shaped with a thick outer tangential wall. The major portion of the secretion product is transported to the canal cells through plasmodesmata. The stigmatic papillae lack the secretory zone. The stigmatic exudate appears before pollination. The stigmatic exudate is transported through intercellular spaces. The styler canal is filled with aqueous solution containing high molecular weight protein and polysaccharides - galactose, arabinose, rhamnose, glucuronic acid. The exudates also play a role in protecting the fragile pollen tubes during their growth.

In closed type of styles, a compact core of transmitting tissue is present. It consists of thin walled cells having plasmodesmata. The innermost wall layer is composed of pectic substances and hemicelluloses. As the pollen tube grows down the style, the intercellular spaces become filled with secretion products

Terminal Questions

1. i) self- pollination and cross pollination

Self-pollination - This involves the transfer of pollen from anther to stigma of the same flower or of another flower on the same plant.

Cross-pollination - This involves the transfer of pollen from one flower to the stigma of a flower of another plant of the same species. If pollination occurs between the two flowers of the same plant, it is called as geitonogamy, and if it is between the two flowers on different plants, it is referred as xenogamy.

- ii) hydrophily and zoophily

Hydrophily - It is also referred as water pollination. It occurs in aquatic plants having flowers at the surface of water or under the water. Example- *Vallisneria*, sea grasses such as *Phyllospadix* and *Zostera marina*.

Zoophily - It is also called as animal pollination. The pollinators mainly include marsupials, primates, and rodents. African lily, *Massonia depressa* is pollinated by rodent species from the South African region. Animal pollinators get benefitted by getting nectar.

iii) wet and dry stigma

Wet stigma- it is covered by sticky secretion. It is found in *Aegle marmelos* and *Petunia hybrida*. The exudates are secreted by stigmatic papillae. The exudate is highly viscous, refractive and adhesive. The exudates contain lipids, proteins, glycoproteins, carbohydrates (glucose, fructose, and sucrose), amino acids and phenols.

Dry stigma- it lacks any secretion. Example-cotton. It is covered with long unicellular hairs which show distinct and continuous cuticle closely pressed to the wall.

iv) Psychophily and hymenophily

Butterflies help in the pollination of flowers that produce colorful, showy fragrant flowers. The flowers have long nectar filled tubes. This type of pollination is called as **Psychophily**.

Bees pollinate showy, colorful and fragrant flowers. The nectar guides function to attract and guide bees. Bee pollination is also referred as **Hymenopterophily or Melittophily**.

2. *Salvia* species is pollinated by bees. The plant exhibits a specialized 'turnpipe' floral mechanism. The corolla is bilipped and the stamens are attached to the corolla tube. Half of the anther is fertile and other half is sterile. The sterile portion joins together to form the sterile plate placed above the lower lip of the corolla. When bee visits the flower for nectar it pushes the sterile plate. This brings down the fertile anthers on its back dusting it with pollen. When the bee visits another flower, the forked stigmas pick up the pollen from the back of the insect.
3. The exudates present on the stigma contain lipids, proteins, glycoproteins, carbohydrates (glucose, fructose, sucrose), amino acids and phenols. The lipids protect stigma from desiccation and regulates the availability of water to the pollen. The phenolic compounds protect stigma from insects and other pests. The enzymes diffusing out of pollen grains on the stigma release free sugars from phenolic glycosides which provide proper osmotic conditions. The sub epidermal cells elongate to form secretory zone with large schizogenous cavities filled with lipid secretion. The cells contain numerous amyloplast and large amount of lipid globules. The lipid exudates accumulate between the cell wall and the cuticle.

Acknowledgments

Fig.10.8 : Pollination by humming bird
(<https://www.google.co.in/search?q=pollination+by+humming+bird>)

UNIT 11

FERTILIZATION

Structure

11.1	Introduction	11.6	Incompatability
	Objectives		Types of Incompatibility
11.2	Pollen Germination		Intraspecific Incompatibility
	Pollen-Stigma Signaling		Interspecific Incompatibility
11.3	Pollen Tube Structure and Growth	11.7	Genetic Basis of Self-Incompatibility
	Pollen Tube Structure		Factors Involved in Incompatibility
	Pollen Tube Invasion	11.8	Methods to Overcome Incompatibility
	Pollen Tube Entry into Ovule		Significance of Incompatibility
11.4	Syngamy and Triple Fusion	11.9	Summary
11.5	Barriers to Fertilisation	11.10	Terminal Questions
		11.11	Answers

11.1 INTRODUCTION

In unit 9 you have studied about the development of male and female gametophytes. You have also studied about the formation of gametes through gametogenesis, development of sperms in male gametophyte and egg in the female gametophyte in unit 9. You may recall that as a result of meiosis, haploid pollen grains and egg cell are formed. The next two vital steps are pollination and fertilization. With pollination begins the process that leads to transfer of male gametes to embryo sac located in the ovule. Pollen stigma interaction plays a significant role in sexual reproduction. The pistil develops mechanisms to recognise compatible pollen and permit growth of pollen tubes through style and stigma. Pollination initiates many changes in the pistil. These changes play an essential role in germination of pollen and in pollen tube growth. Some factors in the stigmatic surface prevent the foreign or incompatible pollen from germinating and forming the pollen tube thus causing a rejection reaction. In this unit you will study various developmental events during pollen tube growth, features of pistil, pollen-pistil interaction, double fertilization, incompatibility and various methods to overcome incompatibility.

Objectives

After studying this unit you would be able to :

- ❖ enlighten by the process of pollen germination and pollen tube growth;
- ❖ describe the mechanism of entry of pollen tube in embryo sac;
- ❖ explain various ways by which fertilization fails;
- ❖ explain and describe syngamy and triple fusion;
- ❖ describe different types of incompatibility;
- ❖ identify various methods to overcome incompatibility; and
- ❖ emphasise the significance of incompatibility.

11.2 POLLEN GERMINATION

Alignment of the microspores within the callose wall is important for maintaining polarity of vegetative and generative cells release of pollen as a monad, tetrad, or cluster, and exine sculpturing and aperture position. A primexine matrix, the precursor of exine, typically is deposited between the callose wall and the plasma membrane of the developing microspores. The callose wall later is degraded, enabling the pollen grains to be released as monads. Lipids are present at the interface between pollen and stigma.

The pollen coat is generated by the tapetal cells that surround developing pollen grains. The pollen coat is largely lipidic and possesses carotenoids as major pigments. Pollen coat also contains volatile lipids such as polyunsaturated C18 free fatty acids. These are known to be potent attractants of honeybee. Cells secrete oil droplets and proteins. Medium, long-chain and very-long-chain fatty acids, proteins, glycoconjugates, carotenoids, and flavonoids have also been found to be present in these cells. Stigma provides conditions for the retention and germination of pollen grains. The pollen grains germinate and form the pollen tubes. The receptivity of the stigma for pollen remains for a short period and varies from species to species. Pollen adhesion, hydration and germination take place on the stigma. Adhesion is determined by the stickiness of the pollen, stigma surface, orientation of the exine, composition of the pellicle, electrostatic forces and specificity between the two parents.

At maturity, secretions and enzymes present on the receptive surface of a stigma help in the process of pollen germination and guidance of pollen tube into the style. The stigmatic surface helps in: (1) pollen adhesion, a process that is independent of protein–protein interactions but involves the role of pollen coat proteins; (2) pollen hydration, a process by which lipids modulate water transport and proteins mediate pollen incompatibility reaction; (3) pollen activation and germination- a process that depends on polarising cues, cytoplasmic rearrangements, and the harnessing of biomechanical forces for the focused breaching of the exine wall; and (4) stigma invasion, a process that involves the activities of digestive enzymes. At maturity, the pollen

covering can be divided into three principal strata, with the relative amount of each varying between species: (1) an outer exine wall, itself multilayered, composed of the chemically resistant polymer sporopollenin and interrupted by openings called germ pores or apertures; (2) an inner intine, also sometimes multilayered, made primarily of cellulose; (3) a pollen coat, composed of lipids, proteins, pigments, and aromatic compounds, that fills the sculptured cavities of the pollen exine. It has been indicated that adhesion of pollen on stigma is dependant on exine (Fig 11.1).

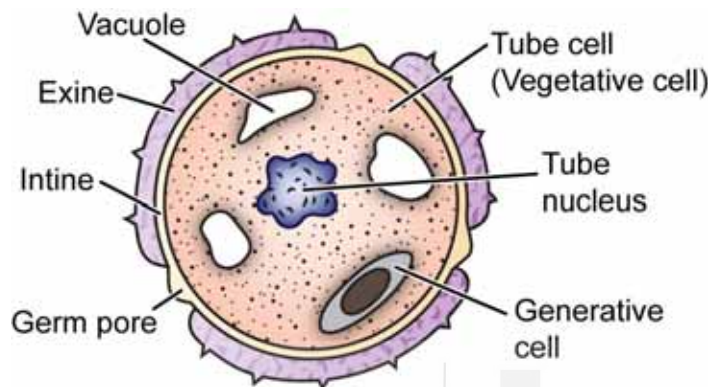


Fig. 11.1: Structure of mature pollen grain.

Moisture present on the stigma helps in the hydration of the pollen. Hydration transforms a pollen grain from a nonpolar cell to a highly polarised cell. The process is referred as pollen polarization. The stigma and pollen coat provide lipids, as medium of water uptake in pollen hydration. In some species in addition to lipid, glycine rich protein from pollen coat also plays a role in pollen hydration. In stigma type of Solanaceae, stigmatic lipids are found to be essential for pollen tube growth toward the style by creating a gradient of water. Hydration triggers the release of pollen wall proteins and subsequent interaction (compatibility) between the two parents. Most pollen grains after the release from anthers possess about 15 to 35% water content. When pollen grains reach wet stigma, they immediately get surrounded by water whereas those present on the dry stigmas mobilise their lipid-rich pollen coat to form an interface between the two cell surfaces. This interface promotes water flow. Nutrients and other small molecules are transported rapidly into the grain from the stigma exudates (wet stigmas) or stigmatic papillae (dry stigmas) by different mechanisms.

In plants with dry stigmas, pollen hydration provides an effective early barrier to incompatible pollination. The stigma can hydrate a compatible grain, while restricting the hydration of foreign or incompatible pollen. The stigmatic exudates support extension of pollen tube by providing nutrients. These changes occur within minutes after hydration and include the formation of filamentous cytoskeletal structures present around the nuclei, actin cytoskeleton polarisation toward the site of tube emergence, reorientation of the large vegetative nucleus so that it enters the extending tube before the generative cell, assembly of mitochondria and polysaccharide particles at the site of the elongating tube tip, and selection of the pollen plasma membrane for secretory vesicle targeting and deposition of callose (β -1 $\rightarrow$ 3 glycan) at the site of tube emergence.

In most of the dicot plants such as cotton, after the pollen germinates on the stigma the pollen tube grows on the surface of the stigmatic hairs. The tube growth is continued through thick wall layers of the transmitting tissue of style. The cells become thicker due to dictyosome activity. Callose is deposited in the pit fields on the transmitting tissue after the passage of the pollen tube (Fig. 11.2). This changes the permeability of the cells. Callose is formed as the result of wounding and as a response against cell leakage.

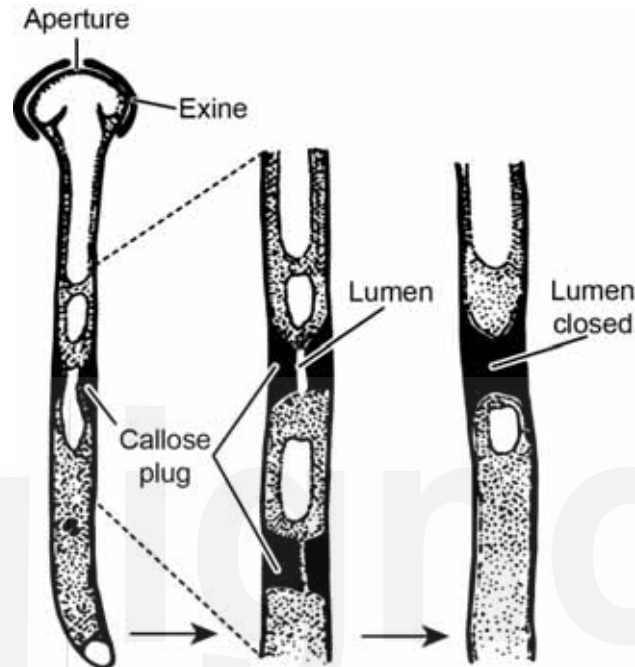


Fig. 11.2: Callose plug formation in pollen tube.

In plants having dry stigma, the cuticle provides a physical barrier for the entry of the pollen tube. The activation of the enzyme cutinase breaks the cuticle. The enzyme cutinase released by the pollen tube digests the stigmatic cuticle. The tube penetrates the pectocellulosic wall of the papillae and traverses the intercellular spaces of the stigma and style. Finally it passes through the intercellular matrix of the transmitting tissue. In plants with dry stigma and hollow style, the cuticle over the papillae is continuous throughout the stylar canal and subcuticular mucilage. In plants with wet stigma and closed style, the pollen tube enters the intercellular matrix of the stigma before entering the style. The pollen tube enters through the intercellular spaces or thick wall of the transmitting tissue and grows through the intercellular matrix of the stigma and style. The pollen tube grows on the surface of the stigma and enters the stylar canal cells. In plants with hollow style the pollen tube enters through the stylar canal and grows along the surface of canal cells. The tubes come in contact with the extracellular components secreted by the epithelial cells lining the stylar canal. The tubes grow further into the ovary along the placenta and draw water and nutrients from the surrounding tissues. In two-celled pollen systems, pollen tube growth occurs in two phases- a period of slow growth in which pollen reserves are utilized and a period of rapid growth during which the nutrients from style are utilized by the pollen tube. However, in three-celled systems pollen grain - a short lag period (phase between hydration and germination) and a faster rate of pollen tube growth as compared to two-celled pollen grain systems is observed (Fig. 11.3).

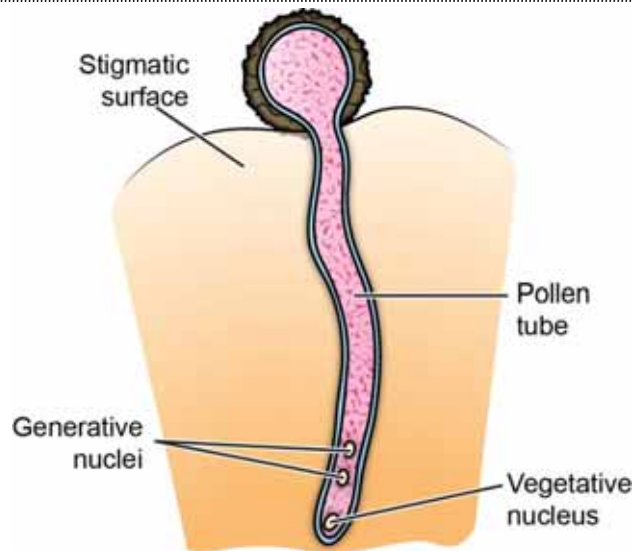


Fig. 11.3: Germination of pollen and formation of pollen tube.

Pollen grains contain auxins and gibberellins. These hormones play a role in activation and enlargement of the ovary following pollination. Pollen from the unrelated species, non viable pollen or pollen extracts can prevent abscission and cause swelling of the ovary and formation of normal but seedless fruits. Some amount of auxin or auxin- like substances and gibberellic acid (GA) supplied by germinating pollen initiate minimal growth and metabolic processes in the pistil. The activation of enzymes liberates additional amounts of auxin from the tissue of the style and ovary. The auxin initiates growth in the fertilized ovules. The supply of auxins and gibberellins from germinating pollen triggers the development of fruit and is responsible for the subsequent release and production of additional amounts of plant growth regulators in the pistil tissues. In some plants, there is a marked decrease in the oxygen tension during pollen tube growth. The oxygen concentration further drops with the progress in the growth of the pollen tube. The level of oxygen is restored after the passage of the tube. The tube grows aerobically through the stigmatic and styler tissues.

The conditions for growth of the pollen tube are provided by pistil. The nutritional requirements of pollen germination and tube growth can be studied under *in vitro* conditions, the pollen tubes can grow as long as few mm to 1 cm. The length of the pollen tube in lily is 10 times more than width. Hormones and amino acids stimulate the pollen tube growth under *in vitro* conditions.

The factors required for pollen germination and tube growth include:

- high relative humidity
- Carbohydrates - sugars control osmotic pressure and serve as respiratory substrates.
- Boron - It prevents bursting of pollen tube and helps in the translocation of sugars. It has direct or indirect effects on the enzyme activities involved in the biosynthesis of carbohydrates.
- Calcium - The growth of the pollen tube is vigorous and rigid. Calcium antagonises the inhibitory effects of heavy ions. The effect of the calcium is regulated by osmotic milieu, oxygen and borate. The effect of calcium is enhanced by inorganic ions such as Mg, K, Na and H. Calcium is also required for wall synthesis.

- Enzymes - cellulose, pectinase, callase present in pollen grains increase the rate of pollen tube elongation. Digestive enzymes such as amylase and lipase help in utilizing stored food in pollen and pistil.
- Growth regulators - auxins and gibberellins promote pollen tube growth and elongation.
- Media composition - Elements such as sucrose, borate (H_3BO_3), calcium (CaNO_3), magnesium (MgSO_4) and potassium (KNO_3) assist in growth of pollen tube. In lily cobalt accumulated in the pistils was found to be crucial for the growth of pollen tube. Cobalt is found to activate aminoacylase found in the pollen.
- Physical factors such as temperature regulate pollen tube germination. You will also do a practical in pollen germination in BBYCL-136 and can see the germination of pollen grains.

11.2.1 Pollen-Stigma Signaling

After hydration, germination occurs in which the pollen tube grows into the cell wall of the stigmatic papillae toward the stylar tissue. Germination is mainly regulated by Rop, a member of GTPase - large family of hydrolase enzymes that can bind and hydrolyze guanosine triphosphate. Research findings indicate that over expression of Rop1 in wild type helps in pollen germination. while rop1 mutant shows inhibited pollen germination. After germination on the stigma, pollen tubes initiated in an extra cellular matrix (ECM) depend upon secretions of both pollen coat and stigmatic exudates. The pollen tubes must be guided in the style. The stigmatic secretion of lily is involved in pollen tube guidance in the style. Fertilization in flowering plants is a coordinated event and sperm cells devoid of flagella are carried by directional growth of the pollen tube toward the ovule and embryo sac. Signaling network regulating pollen tube growth and development in flowering plants is highly conserved. Regardless of the type of stigma, pollen-stigma interactions in general require lipid at the pollen-stigma interface, water as initial directional cue for pollen tube growth, and small cysteine-rich proteins are involved.

SAQ 1

- a) Fill in the blanks :
- The polarity of vegetative and generative cell is regulated by alignment of within the callose wall.
 - is the precursor of exine.
 - Pollen coats contain lipids.
 - Stigma provides conditions for the retention and of pollen grains.
 - wall is multilayered and composed of the chemically resistant polymer sporopollenin.
 - is a layer composed of lipids, proteins, pigments, and aromatic compounds and fills the sculptured cavities of the pollen exine.

- b) Answer in one word :
- i) Adhesion of pollen on stigma is dependant on this layer.
 - ii) The process that triggers the release of pollen wall proteins and subsequent interaction (compatibility) between the two parents.
 - iii) The substances that support extension of pollen tube by providing nutrients.
 - iv) The substance that gets deposited in the pit fields on the transmitting tissue after the passage of the pollen tube.
 - v) The enzyme released by the pollen tube that digests the stigmatic cuticle.
 - vi) The pollen tube enters through this region of the transmitting tissue.
 - vii) These hormones play a role inactivation and enlargement of the ovary following pollination.
- c) How stigma helps in the process of pollen germination and guidance of pollen tube into the style?
- d) 'Hormones support pollen germination and pollen tube growth'. Explain the statement.

11.3 POLLEN TUBE STRUCTURE AND GROWTH

After reaching the stigma, the pollen germinates. The germinated pollen grain forms the pollen tube through which the male gamete enters the ovary. Stigmas cannot discriminate different pollen grains but promote outcrossing or self-fertilization by coordinating the timing of their maturation followed by release of pollen. Stigma maturation and receptivity depend upon enzymatic activity which supports pollen germination and tube growth. Stigma development is linked to the development of other floral organs. Mature stigmas appear to be able to promote pollen tube growth while the immature pistil is unable to support tube navigation to the ovules.

11.3.1 Pollen Tube Structure

The pollen tube wall shows two distinct regions in the stigma and style. Outer tube wall is thin and composed of cellulose. Inner portion of the wall is thicker, homogeneous and rich in callose. The substances required for pollen tube wall formation is contributed by the polysaccharides present in the pistil tissues. The polysaccharide component of the stigmatic exudates gets incorporated in the cytoplasm of the growing pollen tubes and some part of the exudates is metabolised before being utilised for pollen tube wall biosynthesis. A large amount of new pollen wall material is synthesised during growth (Fig 11.4).

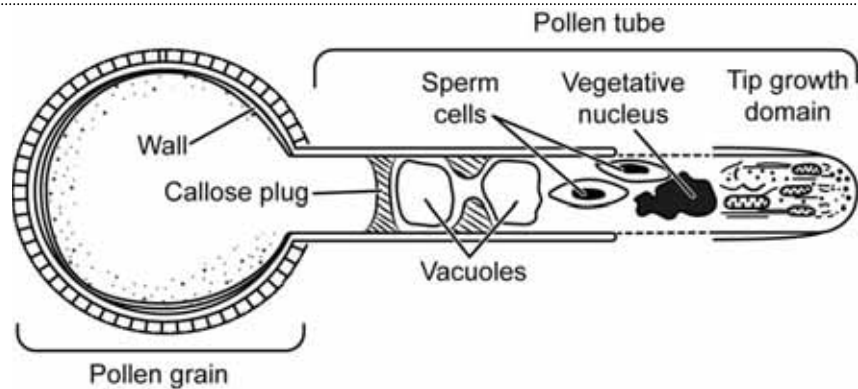


Fig 11.4: Structure of pollen tube.

The tip of the pollen tube is rich in RNA and proteins. The zone has numerous vesicles and network of smooth membranes. The vesicles fuse with each other. The vesicles contain pectin and the RNA residues. The dense cytoplasm of the pollen tube contains numerous mitochondria, dictyosomes, ER, ribosomes, and poorly developed plastids. Behind the tip region, pollen tube possesses less dense cytoplasm and more dispersed organelles. In this region amyloplasts are present. Large vesicles produced by dictyosomes fuse with the plasma membrane of the pollen tube. With the growth of the pollen tube, the cisternae of the ER become narrow indicating that protein is gradually utilised during the tube growth. In the older parts of the pollen tube, callose plugs are formed that separate the richly cytoplasmic tip region from the vacuolated distal part (Fig. 11.5).

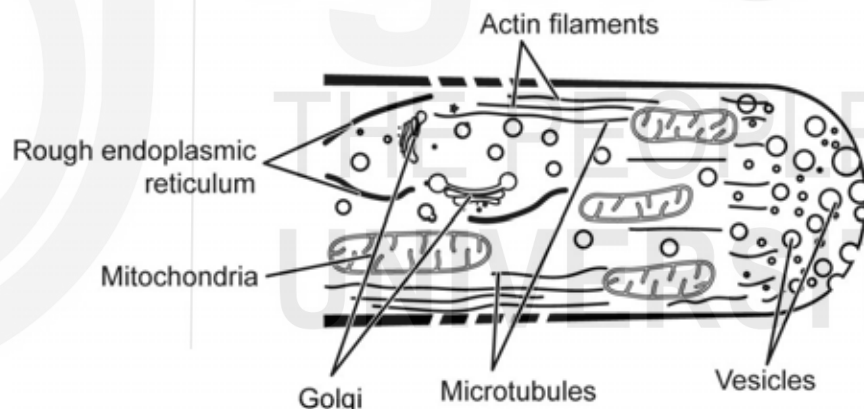


Fig. 11.5: Enlarged structure of pollen tube tip.

Incompatible and compatible pistils show significant differences in the structure of the pollen tube. Pollen tubes growing in the compatible pistils show deep embayments but no compartments. The tube undergoes transition from the autotrophic nutrition derived from food stored in pollen (characterised by compartmented cap of golgi derived vesicles) to heterotrophic condition (received from pistil). The secretion products from the stylar canals enter the pollen tube through deep embayments (tubes growing in incompatible pistils are not able to make this transition and thus are not able to continue growth due to exhaustion of endogenous food reserves).

11.3.2 Pollen Tube Invasion

The path of the pollen tube germination and its penetration in the stigmatic tissue is determined by the nature and structure of the cell walls, morphology and distribution of the pistil tissues. The stylar tissue provides nourishment to

the pollen tubes. In *Petunia* and *Oenothera*, the pollen tubes derive nourishment (sugar and amino acids) from the stylar tissue. The growth of the tubes is accompanied by the inflow of carbohydrates into the pistils. In *Aegle marmelos* the cells surrounding the stylar canals show presence of starch before pollination. Subsequently the starch gets digested; the canal cells and the basal portion of the stigmatic papillae show the presence of reducing sugar. The sugar also gets used up during pollination and passage of pollen tube. This has been noted in species of *Fritillaria*, *Pavonia* and *Zephyranthes*.

Extracellular proteins contributed by tapetum assist in pollen germination, penetration of the pollen tube and its growth. Some proteins are involved in the recognition responses and control inter and intra specific incompatibility. Gametophytic proteins are injected into the intine from the microspore cytoplasm and are involved in the germination and nutrition of the grain in gametophytically controlled incompatibility systems.

After emerging from the exine wall, pollen tubes can only enter the style after transiting the stigma barrier. In plants with open styles, the stigma is covered with an epidermis that is continuous with the style, while in species having closed styles, pollen tubes grow through the outer cuticle and cell wall of the stigma papillae to enter the style. Enzymes secreted by pollen play an important role in pollen tube invasion of the stigma surface. Enzymes such as acid phosphatase, ribonuclease, esterase, amylase and protease are present mainly in the intine of pollen grains and tubes. Esterase enzyme is commonly known as cutinase. It is supposed to play a crucial role in breaching the cuticle of stigma. These enzymes have been identified in the pollen of *Brassica*, *Tropaeolum* and many other taxa. In some species, proteinaceous pellicle of dry stigma surface also helps in removal of cuticle. Removing or disrupting the pellicle prevents compatible pollen tubes from entering the stigma, despite normal germination.

11.3.3 Pollen Tube Entry into Ovule

The process of entry of pollen tube into the ovule reach to the embryo sac has been categorized into three types :

Porogamy - In this method, the pollen tube enters the ovule through the micropyle (Fig 11.6). This mode of entrance is referred as porogamy and fertilization is referred as porogamic fertilization. This has been reported as the most common mode of pollen tube entry in plants. This type of pollen tube growth is chemotropic i.e. guided by some chemical stimulus. It has been suggested that some substances secreted by the filiform projections of the synergids are responsible for porogamy.

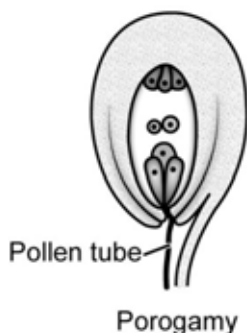


Fig 11.6: Porogamy.

This type of pollen tube growth in some species (e.g., *Euphorbia*) is facilitated by special structure called obturator (Fig 11.7). It is a swelling of placenta or funiculus and functions like a bridge for the pollen tube to enter the micropyle.

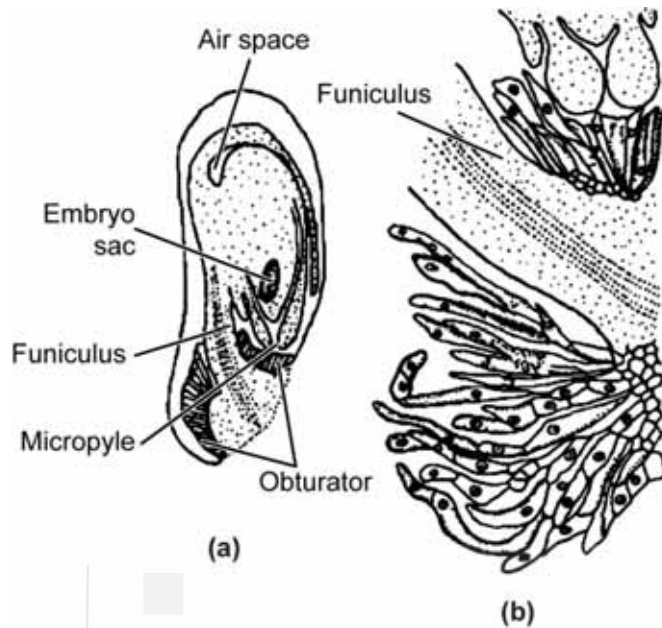


Fig. 11.7: Structure of an obturator.

Chalazogamy - in this type, the pollen tube enters through the chalazal end of ovule to reach the embryo sac and the process is referred as chalazogamy (Fig.11.8) e.g., *Casuarina*, *Juglans*, *Betula*.



Fig 11.8: Chalazogamy.

Mesogamy - In this type the pollen tube enters through the integuments to reach the embryo sac, the process is referred as misogamy and the mode of fertilization is referred as mesogamous. The pollen tube enters the ovule through the side of the integuments near to the placenta e.g., *Cucurbita*, *Populus*. Irrespective of the point of entry in the ovule, the pollen tube then finds its way to the egg apparatus of embryo sac (Fig. 11.9).

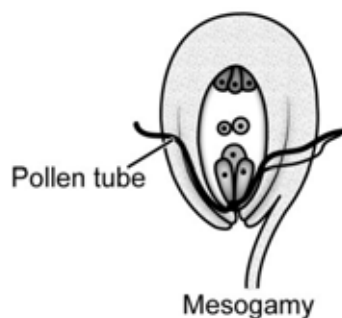


Fig. 11.9: Mesogamy

The egg apparatus, having two synergids and the egg, is the specialized structure meant to achieve double fertilization characteristic of angiosperms (Fig 11.10). In most of the investigated species, one of the two synergids begins to degenerate before the arrival of pollen tube. This degenerated synergid receives the pollen tube. The pollen tube discharges its contents i.e., two sperm cells, vegetative nucleus and some cytoplasm into one of the synergids via a pore at the tip or through a subterminal pore.

Studies have shown that pollen tube enters at the apical region of the filiform apparatus, grows through it and reaches the cytoplasm of the synergid (Fig. 11.10). Synergids play an important role in determining the entry of pollen tube in the embryo sac. They secrete some chemotropic substance that attracts the pollen tube. They also affect the dissemination of male gametes in the embryo sac. One of the male gamete nucleus is transferred to egg for syngamy, and the other male gamete shifts to the central cell for triple fusion. Studies revealed that male cytoplasm is also involved in fertilisation. Synergids are supposed to function in three ways :

- they secrete some substances which exert a chemotropic influence over the pollen tubes and direct their growth
- the degenerating synergids forms the seat for pollen tube discharge in the embryo sac.
- Synergids also have nutritive role for embryo sac. They absorb nutrition from nucleus and pass it on to central cell.

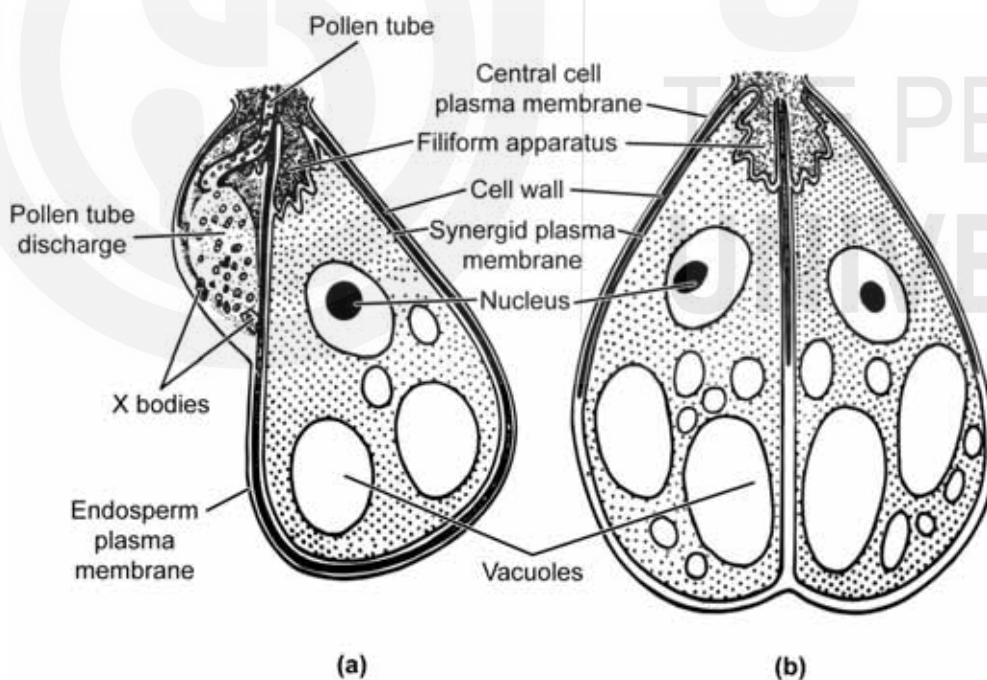


Fig. 11.10: Structure of ovule showing filiform apparatus and entry of pollen tube.

Thus, for double fertilization:

1. One of the two synergids which is supposed to receive pollen tube degenerates even before the entry of the pollen tube in the embryo sac. The degeneration starts after pollination. It is marked by the reduction in size, disappearance of plasma membrane, disorganisation of organelles and distortion of vacuoles (Fig. 11.10).

2. Pollen tube penetrates the embryo sac wall, grows between the synergids and then enters the degenerating synergid through its filiform apparatus (Fig 11.11).

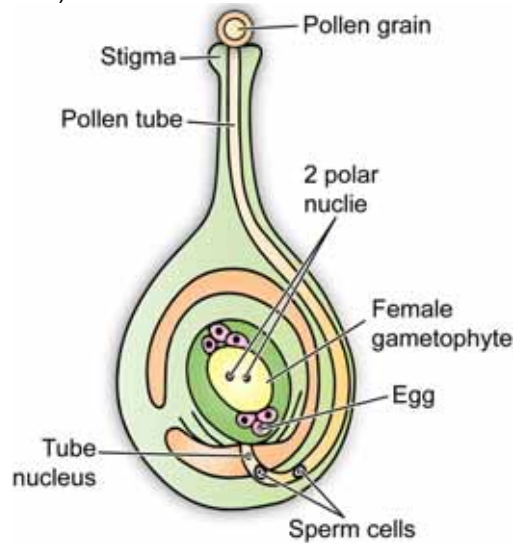


Fig. 11.11: Diagrammatic view of Fertilization.

3. After growing through the filiform apparatus, the pollen tube enters the cytoplasm of the degenerated synergid. A pore develops either at the subterminal position or at the tip of the tube. Both the sperms (male gametes) along with some part of the cytoplasm are released in the synergid. The discharge of contents into the synergids occurs within a few seconds of the pollen tube entry. The other synergid also degenerates after sometime. In some investigated species, both the synergids remain intact at the time of pollen tube entry. The synergid that receives the pollen tube starts degenerating first, followed by the other (Fig 11.12).

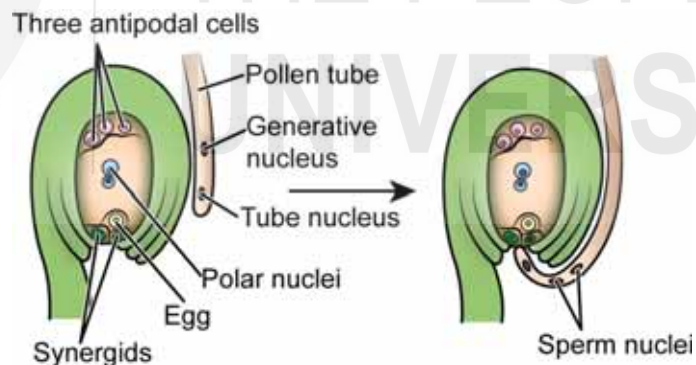


Fig. 11.12: Process of Fertilization (Diagrammatic).

In some plants three celled pollen is noted. In this case, the sperms are dimorphic. One sperm is larger and contains abundance of mitochondria. The other sperm is smaller and contains plastids e.g., *Plumbago zeylanica*, *Brassica oleracea* and *Spinacea olearacea*. The sperms are linked by a common cross wall or evagination of the plasma membrane. One of the sperm gets connected to the vegetative nucleus by a cytoplasmic extension, thus forming a three celled male germ unit (MGU). The organisation of the cells in the MGU is fixed and determines which sperm will fuse with the egg, and which one will participate in triple fusion. In *Plumbago* the mitochondria- rich larger sperm connected with the vegetative nucleus fuses with the egg, while the smaller sperm without any contact with the vegetative nucleus participates in triple fusion. In species having two - celled pollen, the MGU is organised in

the pollen tube following division of generative cell to form two male gametes e.g., *Petunia*, *Gossypium*. In some species, the MGU appears only after pollen germination. The MGU travels through the pollen tube as one entity but after reaching the ovule, the vegetative nucleus separates from sperms followed by separation of two sperms from each other. The spatial arrangement of the sperms in the MGU allows fusion with the egg and secondary nucleus.

In some embryo sacs as in *Plumbago*, the synergids are absent and the pollen tube penetrates the embryo sac in between the wall of the embryo sac and egg cell. One of the male gametes moves towards the egg and other to the polar nucleus. One of the sperm fuses with the egg.

SAQ 2

- a) State whether the statements are 'True' or 'False' :
- The polysaccharide component of the stigmatic exudates gets incorporated in the cytoplasm of the growing pollen tubes.
 - In the older parts of the pollen tube callose plugs are formed that separate the richly cytoplasmic tip region from the vacuolated distal part.
 - Incompatible and compatible pistils do not show any differences in the structure of the pollen tube.
 - The method in which the pollen tube enters through the integuments to reach the embryo sac, is referred as porogamy.
 - In most of plant species, one of the two synergids begins to degenerates before the arrival of pollen tube.
 - The organisation of the cells in the MGU is fixed and determines which sperm will fuse with the egg and which one will participate in triple fusion.
- b) Fill in the blanks:
- enzyme is supposed to play a crucial role in breaching the cuticle of stigma.
 - In the pollen tube enters through the chalazal end of ovule to reach the embryo sac.
 - The pollen tube enters at the apical region of the apparatus, grows through it and reaches the cytoplasm of the synergid.
 - play an important role in determining the entry of pollen tube in the embryo sac.
 - Synergids secrete some substance that attracts the pollen tube.
 - One of the sperm gets connected to the vegetative nucleus by a cytoplasmic extension, thus forming a three celled structure called
- c) What roles do synergids play during pollen tube growth and fertilization?

11.4 SYNGAMY AND TRIPLE FUSION

One of the two sperms or the male gametes fuses with the egg to form zygote (progenitor of next generation). The process is called **syngamy**. The other sperm fuses with the secondary nucleus or the polar nuclei resulting in formation of the primary endosperm nucleus. The process is referred as **triple fusion** (one haploid male gamete and two haploid polar nuclei = $3n$). Such a process of fusion of gametes is called **double fertilization** (Fig. 11.13). It is an important feature noted in angiosperms. The phenomenon of double fertilization was discovered by Nawaschin in 1898 in *Lilium* and *Fritillaria*.

Syngamy

One of the sperms discharged in the the synergids enters the egg. The male gamete comes in contact with the plasma membrane of the egg. The membranes of the sperm fuse with that of the egg, forming a bridge through which the male nucleus enters the egg cytoplasm. Nuclear fusion is mediated through the fusion of outer nuclear membranes of the two nuclei. The inner membrane also fuses at localised areas forming a small bridge between two nuclei. Enlargement of the bridge causes fusion of male and egg nuclei (syngamy). Syngamy leads to formation of zygote - the progenitor of embryo.

Three types of syngamy have been identified depending upon the mitotic stage of male gamete at the time of nuclear division (Gerassimora-Navaschina, 1932).

- a) Pre-mitotic type - the male nucleus fuses with the egg before reaching the mitotic interphase. The sperm nucleus attains its mitotic rest after penetrating the egg nucleus but prior to the initiation of the first zygotic division. This type of fusion has been noted in Poaceae, Ranunculaceae, Solanaceae.
- b) Post mitotic type - the male nucleus undergoes interphase. Its contact and fusion with the egg is postponed until the initiation of first mitosis in zygote. In this type of syngamy the sperm nucleus completes its mitotic cycle while in contact with the female nucleus. The fusion occurs only after the commencement of the first mitosis in zygote. It is also known as zygotic mitosis. It has been noted in *Lilium*, *Fritillaria*.
- c) Intermediate case - the fusion occurs when male nucleus is in interphase. The male cytoplasm does not participate in fertilisation. The sperm nucleus fuses with the egg nucleus after completing telophase of its previous mitosis e.g., *Imaptiens*, *Mirabilis*, *Tradescantia*.

Triple fusion

The fusion of the male gamete with secondary nucleus occurs in the same pattern as syngamy. A single polar nucleus (n) from the micropylar end and a single polar nucleus (n) from the chalazal end migrate towards the center of the embryo sac and fuse to form the diploid secondary nucleus ($2n$). During the process of double fertilisation, one male nucleus fuses with the secondary nucleus to form triploid primary endosperm nucleus ($3n$). This is referred as triple fusion (Fig. 11.13). The syngamy normally happens earlier than triple fusion. The central cell with triploid nucleus is the seat of formation of endosperm.

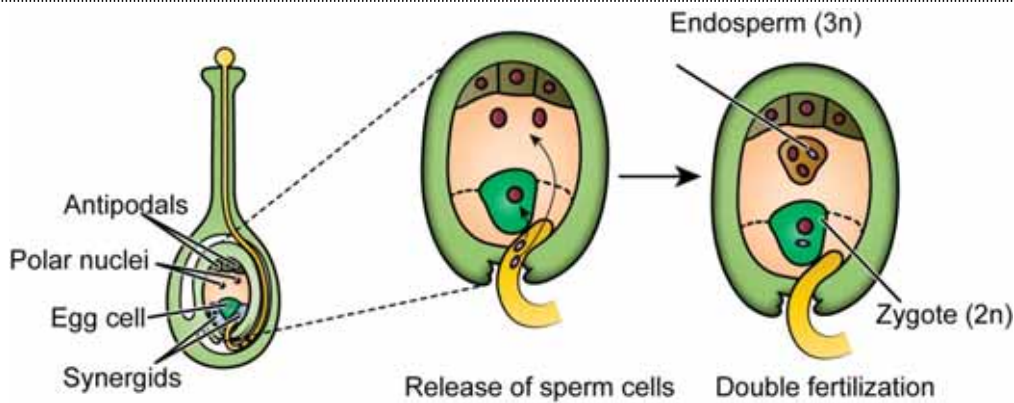


Fig. 11.13: The process of double Fertilization (Diagrammatic).

SAQ 3

Define the following terms :

- Porogamy
- Triple fusion
- Syngamy

11.5 BARRIERS TO FERTILISATION

The barriers to fertilisation may be on the stigma or in the style, at any level from pollen germination to the discharge of male gamete to reach the egg. On the other hand, the syngamy barriers include the inhibition of the entry of the pollen tube to the ovary, ovule or within embryo sac. During pollination, the barriers of fertilisation are morphological and ecological. The physiological and biochemical processes involved in the sexual incompatibility have been recognised. These include two reactions.

Recognition reaction - The compatibility has been decided at the molecular level by the pistil. In SSI systems, the acceptance or rejection of the pollen is decided by the stigmatic surface. In GSI systems this happens mostly in style.

Rejection reaction - The physiological and biochemical processes are set in the pistil by the recognition reaction specific to the type of pollen grains that land on the stigma. In SSI (sporophytic self- incompatibility) inhibition can occur on stigma itself thus preventing the germination of pollen or its entry into style. In GSI (Gametophytic self- incompatibility) system, it occurs in style leading to inhibition of pollen tube growth or its bursting.

The pollen wall and its proteins play an important role in pollen stigma interaction. Cytochemical studies indicated that pollen wall is perforated by protoplasmic strands which help in the exchange of substrates. Incorporation of proteinaceous substances occurs in the exine and intine. The mobile proteins of the pollen grains are S gene specific. The recognition proteins are held extracellularly in the pollen wall. In GSI systems, these proteins are present inside the intine wall, while in SSI systems they occur in exine.

Exine layer - It consists of two layers. Inner layer is nexine which is continuous and outer is sexine which is sculptured. The outer layer has ornamentations comprising of rod-like baculae showing terminal extensions which fuse to form a roof-like tectum perforated by micropores. During pollen development, tapetum releases tryphine consisting of carotenoid, lipid droplets and fibrogranular proteins into the tectal cavities.

The fibrogranular proteins are enclosed in membrane bound cisternae. In tectate pollen the substances released from the tapetum become incorporated on the surface of the pollen. The cisternae (G) membrane ruptures and the protein released through the micropores enter the tectum and accumulate in the interspaces of the baculae.

Exine held proteins exhibit enzymatic activity. The hydration of the pollen releases the proteins held in the pollen wall layers. The proteins (sporophytic) held in the exine layer take less time to pass out in comparison to those released by intine (gametophytic). In SSI (Sporophytic self-incompatibility) systems therefore the rejection reaction is faster (on stigma) than that in the GSI system (in style).

Intine layer - The inner wall of the pollen is formed as the micropores get released from the tetrads. The proteinaceous lamellae are embedded in the matrix of the intine, concentrating around the germ pore. The proteins are contributed by the cytoplasm of the gametophyte i.e., pollen cytoplasm.

11.6 INCOMPATIBILITY

If pollination and pollen-pistil interaction is compatible, all reactions starting from pollen capture, adhesion, pollen grain germination on the stigma, entry of the pollen tube into the ovule and discharge of male gametes in the embryo sac occurs sequentially resulting in double fertilization to form embryo and endosperm. The process eventually leads to the formation of seed. In other cases, self pollen rejection response can occur at any stage between pollen contact to stigma and pollen tube growth in the style which results in failure of fertilization and embryo development. The process in which fertile pollen fails to germinate or the pollen tube fails to reach the embryo sac to affect double fertilization and produce seed is referred to as **incompatibility**. Inability of the plant producing functional gametes to set seed following pollination among flowers of the same plant is called as self-incompatibility. Rejection most commonly occurs on stigma or style. Incompatibility can also result from difference in length of pollen tubes penetrating the style. Inhibition of pollen-tube growth occurs due to reaction between a substance in the pollen tube and a complementary substance in the style. Self-incompatibility enforces outbreeding. It is one of the mechanisms to prevent self-fertilization that can cause inbreeding depression. It is widespread in many families of flowering plants.

11.6.1 Types of Incompatibility

Incompatibility is of two types :

1. **Intraspecific incompatibility** - It occurs between the individual of same species. It occurs when the viable pollen of the same or other individuals

of a species fail to cause fertilization due to a genetic block. It is known as intraspecific incompatibility or self-incompatibility. Most of the plants are fertilized by the pollen of other individuals. The self pollination is also prevented by adaptations in flowers such as dichogamy, herkogamy and unisexuality.

2. **Interspecific incompatibility** - It refers to the incompatibility that occurs between distinct species and this prevents fertilization between the gametes of distinct species.

11.6.2 Intraspecific Incompatibility

Intraspecific or self- incompatibility prevents self-fertilization. Flowering plants have evolved several unique mechanisms for rejecting the self-incompatible pollen. Self- incompatibility is classified as gametophytic and sporophytic.

Gametophytic self- incompatibility (GSI) is common in species with binucleate pollen and wet stigma. This type of incompatibility occurs when pollen tube growth is inhibited in the style. This type of incompatibility is governed by genotype of the pollen (male gametophyte). Example - Poaceae, Liliaceae, Solanaceae, Fabaceae.

Sporophytic self- incompatibility (SSI) is observed in trinucleate pollen and it is characteristic of dry stigma. This type of incompatibility occurs before a pollen tube penetrates the stigma. In this type the genotype of the sporophytic anther tissue which contributes to the pollen wall components controls the incompatibility process. Example - Asteraceae, Brassicaceae and Convolvulaceae.

Self- incompatibility is thus a device to promote cross pollination. It ensures a certain degree of heterozygosity in a population. It is a major factor in the evolution of the species. It is extremely widespread among the flowering plants. Self- incompatibility has also been classified into two types on the basis of morphological features

Heteromorphic type

In this type of incompatibility, same species produces more than one type of morphological flowers. The plants showing such type of incompatibility show differences in floral structure such as length of styles and anthers.

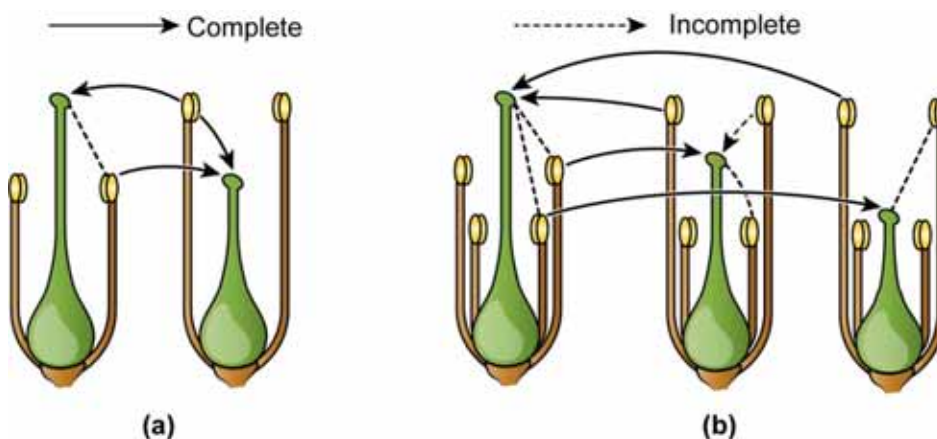


Fig.11.14: Diagrammatic representation of the heteromorphic incompatibility.

a) Distyly; b)Tristyly.

The relative length of styles and stamens in flowers of same species differs. It occurs in a few species only. Individuals of the species are of either two (dimorphic) or three (trimorphic) distinct mating types (Fig. 11.14). This is associated with differences in floral structure such as length of styles and anthers. Each mating type produces only one type of flower. In such forms, the pollen grains from the same individual plant or any other plant bearing the same type of flowers are incompatible. For effective fertilization, pollen grains from any other plant of the same species bearing other type of flowers are required.

Distyly-in this condition seen in *Primula* the plant bears two types of flowers, one (thrum) having a short style and high anthers, other (pin) having a long style and low anthers (Fig. 11.15). Pollen grains from high anthers would fall and stick to stigma of long style of pin flowers, and pollen grains of low anthers would be held on stigma of short styles of thrum flowers. In tristylous flowers of *Oxalis*, there are anthers and styles of three different sizes and pollen grains of any one type can get captured by flowers of other two types.

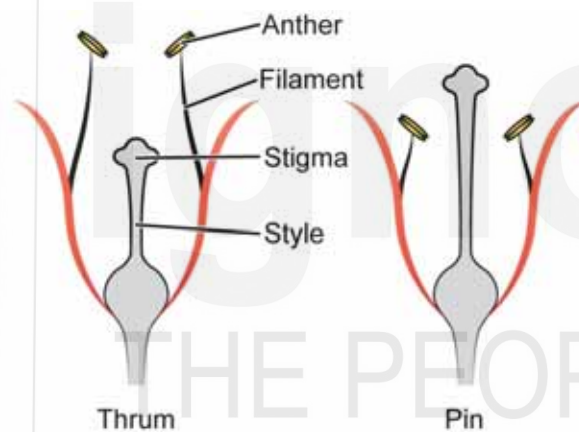


Fig.11.15: Representation of a distyly condition in flowers.

Homomorphic type

This type of incompatibility is characterised by the occurrence of only one type of flowers in all the individuals of a species. There are no floral differences. The condition is characterised by the morphologically indistinguishable mating types.

11.6.3 Interspecific Incompatibility

This type of incompatibility involves prevention of fusion of gametes between members of different species. The rejection reaction can occur at any level. The factors which induce incompatibility factors operate even after fertilization and cause breakdown or failure of embryo development. The non-proteinaceous substances such as phenolics, carbohydrates and proteins present on the surface of stigma might be responsible for this. It is supposed that more than one gene at different loci control this type of incompatibility. In crosses between the incompatible species, fertilization does not occur or the hybrid embryo if formed aborts due to inadequate development of endosperm or lack of support from endosperm. Interspecific incompatibility helps in maintaining the distinction and integrity of species.

Unilateral incompatibility

This relates to incompatibility that operates in one direction. It is ascertained by crossing a self incompatible species and a self compatible species. The self compatible species serves as the female parent. This type of incompatibility operates between species of different genera.

SAQ 4

Choose the correct option :

- a) It occurs when the viable pollens of the same or other individuals of the same species prevent fertilization.
 - i) intraspecific incompatibility
 - ii) interspecific incompatibility
 - iii) homomorphic type of self- incompatibility
 - iv) heteromorphic type of self- incompatibility
- b) The incompatibility is characterized by the occurrence of only one type of flowers in all the individuals of a species and is characterized by the morphologically indistinguishable mating types.
 - i) intraspecific incompatibility
 - ii) interspecific incompatibility
 - iii) homomorphic type of self- incompatibility
 - iv) heteromorphic type of self- incompatibility
- c) This relates to incompatibility that operates in one direction. It is ascertained by crossing a self incompatible species and a self compatible species.
 - i) intraspecific incompatibility
 - ii) interspecific incompatibility
 - iii) unilateral incompatibility
 - iv) heteromorphic type of self- incompatibility
- d) The type of incompatibility involves prevention of fusion of gametes between members of different species. The factors which induce incompatibility factors operate even after fertilization and cause breakdown or failure of embryo development.
 - i) intraspecific incompatibility
 - ii) interspecific incompatibility
 - iii) unilateral incompatibility
 - iv) heteromorphic type of self-incompatibility

11.7 GENETIC BASIS OF SELF-INCOMPATIBILITY

Self-incompatibility (SI) is the most common method adopted by plants to avoid inbreeding due to self-fertilization and promote outbreeding. It is noted in about 60% of flowering plants. It provides a barrier to fertilization by self and self-related pollen in hermaphrodite flowering plants. SI is a genetically controlled pollen-pistil recognition system. The incompatibility of the pollen is determined by two genetically distinct forms of SI. Compatibility is determined by the genotype of the gametophyte or pollen hence referred as gametophytic self-incompatibility (GSI) or by the genotype of the sporophytic tissue of the plant from which the pollen is derived and is called as sporophytic self-incompatibility (SSI). In the gametophytic systems, behavior of the pollen is determined by its own haploid genotype and the developing pollen synthesises its own pollen product, whereas in sporophytic self-incompatibility systems, the self-incompatible behavior of the pollen is determined by the parental genotype (Fig. 11.16).

Most of the taxa possess two-celled pollen (vegetative cell and generative cell). In this type the growth of the pollen tube is inhibited in the style. GSI appears to be the most common mode of SI and has been characterized extensively at a molecular level in the Solanaceae and Papaveraceae. The molecular studies of SSI have been largely confined to species from the Brassicaceae. The taxa having three-celled pollen (one vegetative cell and two gamete nuclei) show SSI in which pollen germination and growth of pollen is inhibited on the stigma.

In some species, the self-incompatibility is morphological i.e. plants produce distinct flowers in such a way that the positions of the reproductive organs within a flower create an extra barrier in addition to the existing barriers within species. In other species, self-incompatibility is caused by the phenotype of the pollen, not morphological character of the flower. The way of rejection of incompatible pollen shows variation among the species. In most of the plants, self-incompatibility is genetically controlled by a number of complex cellular interactions occurring between the self-incompatible pollen and stigma.

Incompatibility is a genetically controlled process. Self-incompatibility response is genetically controlled by one or more multi-allelic loci and involves series of complex cellular interactions between the self-incompatible pollen and pistil. It is generally controlled by one gene (S) but sometimes controlled by two genes (S and Z) with many alleles as noted in Poaceae. In families such as Chenopodiaceae, Brassicaceae and Ranunculaceae it is controlled by 3 or more genes. Self-incompatibility in flowers of the same morphological character is genetically controlled by S-locus. The SI system is controlled by a single Mendelian locus (the S-locus), which is comprised of a number of tightly linked genes, coding for pollen-pistil recognition, which function as a single locus. Variation of alleles at this locus provides distinct mating specificity. The S-locus is comprised of a gene complex that encodes specific proteins involved in recognition and rejection of 'self' pollen. These genes are highly polymorphic.

Immuno diffusion assays have shown presence of unique protein for each S allele in *Brassica*. S allele specific antigens are also detected in diffusates of

intact stigma. The assessment of the F_1 and F_2 generations from the cross $S_2 \times S_2$ have shown that all F_1 progenies showed stigmatic protein concentration intermediate to those of parents, while all F_2 individuals showed low, high and intermediate to those of parents. The studies indicated that the S protein is directly proportional to the intensity of the incompatibility reaction.

S gene is activated before the completion of meiosis in sporophytic systems. The products of both the genes are incorporated in all the microspores. S allele specific substances are produced in the tapetum and then incorporated into the pollen exine. Both tapetal and pollen components are involved in controlling sporophytic incompatibility. In gametophytic systems, the action of S-gene is delayed. As you know that there are four microspores in a tetrad of which two microspores receive the product of one S allele and other two microspores receive another S-allele. Genetic systems of incompatibility between pollen and style include heterogamety which depends upon the genotype of the individual pollen grain, and the other is heterostyly which depends upon the genotype of its parent.

Gametophytic self- incompatibility

In GSI the incompatibility type of the pollen is controlled by its own haploid genotype. Incompatibility is controlled by the single S allele in the haploid pollen grain. Thus a pollen grain will grow in any pistil that does not contain the same allele. 'Opposition S- alleles' hypothesis has been proposed to describe the genetic control of self- incompatibility. According to this hypothesis, a single gene 'S' with several alleles controls the incompatibility reactions. Pollen grains having S allele common to any one of the two alleles present in the pistil will not be functional on that particular pistil. For example, if a plant is having S_1 and S_2 alleles in its sporophytic cell including pistil, two types of pollen half having S_1 and the other half having S_2 allele will be produced during microsporogenesis. Neither of these pollen types (S_1 , S_2) will be functional in this plant because in the stylar cells S_1 , S_2 alleles are present. If this plant is to be pollinated with pollen from a plant of genotypes S_2 , S_3 , pollen carrying only S_3 will be able to bring about fertilization. The one with S_2 allele would be non-functional. The pollen from the plants S_3 S_4 on the pistil of a S_1 S_2 plant will be functional. This is because none of the alleles is common to these two plants. Therefore, S allele of the pollen or male gametophyte controls the incompatible reactions (Fig 11.16).

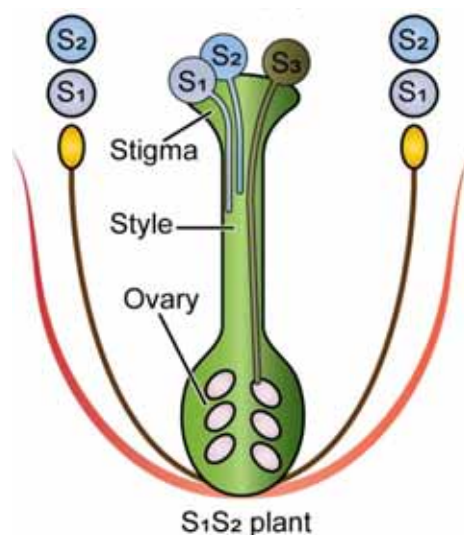


Fig. 11.16: Gametophytic incompatibility.

Sporophytic self- incompatibility

In SSI system, the pollen incompatibility type is controlled by the diploid (sporophyte) genotype of the parental anther in which it was produced. The genetics of SSI was first elucidated in two species from the Asteraceae: *Crepis foetida* and *Parthenium argentium*. Later, SSI was described in species from the Brassicaceae. The SSI is controlled by a single polymorphic locus, S. In SSI, the incompatibility phenotype of the pollen is determined by the diploid parental genome. Hence, pollen grains carry the products of two SI (S) alleles. Diploid sporophytic expression of SI allows dominance interactions to occur between S-alleles and such interactions can occur independently for pollen and stigma. The total number of S- alleles needed to sustain a functional SI system is two, with the one dominant allele exerting its effect on both pollen and stigma. The pollen behavior is same for all irrespective of the S allele they carry. Example- A plant carrying S_1, S_2 alleles would be incompatible to plants having $S_1 S_2, S_1 S_5, S_2 S_4, S_2 S_5$. A plant carrying $S_3 S_4, S_3 S_5$ shows hundred percent compatibility because no allele is common.

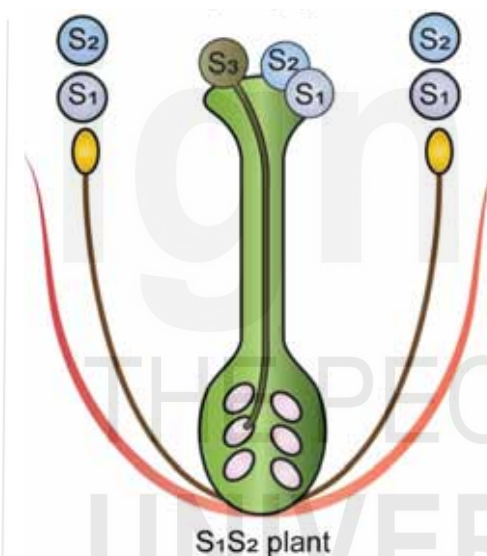


Fig. 11.17: Sporophytic incompatibility.

Heteromorphic incompatibility

It is associated with six pairs of contrasting characters. These are inherited as a single unit. The gene S has been suggested to act as super gene complex with several genes linked to it. Six closely linked genes that determine length of the style (gene G), surface of stigma (gene S), pollen incompatibility (I'), stilar incompatibility (I''), pollen size or shape (P) and stamen height (A) are involved. Heteromorphic incompatibility can further be characterised as sporophytically determined incompatibility reaction. Short style is always dominant for long. The genes and polygenes affect the expression of the S genes.

The distyly condition is controlled by a single gene having two alleles- 'S' for short style dominant and 's' for long style recessive. Long style individuals are therefore homozygous recessive (ss), and short style individuals are heterozygous (Ss). Each type has stamens of two heights corresponding to the height of the stigma in the other two forms. In tristily condition, the plant bears three types of flowers, with long, mid and short styles. The difference in

mating type depends upon the position of stigma and anthers (heterostyly). Two genes M and S control tristily. Gene S is epistatic to gene M. Homozygous recessive of both the genes (ssmm) determine the long style, mid style has ssMM or SSmm, and short styled has genes SSMM, SsMm, SsMM. In both dimorphic and trimorphic forms the sporophytic incompatibility exists. Successful pollination occurs between the flowers having stigma and stamens of the same height. The genetic control is by one gene, S, with two alleles in distyly, and by two genes each with two alleles in tristily.

Homomorphic incompatibility

It is regulated by a single gene 'S' with large number of alleles (multiple alleles). Pollen grains with one S-allele common with one or both S alleles in pistil are incompatible. Pollen is unable to grow in a style which has the same allele as the pollen. The pollen reaction is gametophytically determined and the two alleles in the style are independent. S allele can express a relationship of dominance or independence in pollen or pistil. This type of incompatibility has been recognized in 250 genera belonging to 71 families of angiosperms.

Box12.1: Molecular mechanism known in self- incompatibility.

Some molecular mechanisms are also supposed to regulate the process of self-incompatibility in plants. In members of Brassicaceae sporophytic incompatibility has been noted. A gene product called serinethreonine kinase (SRK) interacts with peptide ligand cysteine-rich protein (SCR), and creates signals that lead to rejection of self pollen. It was indicated that ARC1, a novel U-box protein that binds to the SRK kinase domain, is found to be effector for SRK. It was indicated that a component of hetero-oligomeric protein complex, Exo70A1, interacts with ARC1 which determines the fate of pollen on arrival on stigma. Gametophytic self-incompatibility is widespread system of self- incompatibility. The system in Papaveraceae (poppy family) and the Solanaceae (tobacco family) are examples of gametophytic self- incompatibility. In poppy, small S-proteins secreted by the stigma interact with the S-gene product in the pollen tube, causing the rupture of incompatible pollen. Growing pollen tubes are known to have high concentration of Ca^{2+} . This concentration is rapidly decreased in self incompatible pollen indicating the first signs of arrest of pollen tube. The self- incompatibility in Solanaceae is due to protein from S-gene which is ribonuclease inhibitor that recognizes and inhibits S- ribonucleases in incompatible pollen.

Recognition and rejection reactions on the stigma : The recognition and rejection reactions in SSI system occur on the surface of the stigma placing barriers for pollen germination or penetration of pollen tube into the stigma. In this case, pollen grains either fail to germinate or the small pollen tube formed gets inhibited by callose deposition at the tip. The papillae on the stigma also develop lenticular callose plug after pollination. Inhibition occurs at various levels such as pollen adhesion, hydration, germination and pollen tube entry into the stigma. In some plants, stigma is covered with cuticular layer which the pollen tube fails to penetrate.

In case of compatible conditions, enzyme cutinase breaks the cuticle to allow the growth and entry of pollen tube. The dry stigma possesses a hydrated layer called pellicle over the cuticle. The pellicle layer consists of lipids and

proteins. The pellicle originates from the protrusion on the surface of the papillae through discontinuities in the cuticle. After the pollen is received by the stigma, the papillae cells exude moisture.

In case of incompatibility, the male gametophyte shows number of irregularities such as failure of pollen germination, failure of pollen tube growth, inhibition of one pollen tube leading to emergence of the other one, appressorium like structure at the tip of the pollen tube and/or development of callose plug between the plasma membrane and the pectocellulose layer of the stigmatic papillae

Inhibition reaction between a pollen grain and stigma is localised i.e. it does not interfere with the growth of the other pollen grains present on the stigma. The incompatible pollen shows callose deposition at the rejection site. Exine proteins contribute to incompatibility of pollen. The proteins when isolated and applied directly on stigma give similar response as does the intact pollen.

The pistillate factors responsible for incompatibility are located on the surface of stigma. These include presence of S allele specific proteins on the surface of stigma, specific proteins from the stigmatic leachates. Studies indicated that the recognition factors are synthesised in the stigma during maturation. This type of incompatibility can be overcome by treating stigma with organic solvent, or by bud pollination. This reduces the level of S allele specific proteins or removes them.

Generally the species that shed pollen at two-celled stage show gametophytic incompatibility (inhibition occurring in the style) while those shedding pollen at three-celled stage show sporophytic incompatibility (inhibition occurring in the stigma). Taxa having dry stigma are associated with sporophytic incompatibility, while those having wet stigma are associated with gametophytic incompatibility.

In gametophytic incompatibility, the callose deposition does not occur on the stigma but occurs inside the pollen tube. Sometimes the callose deposition also occurs in the germ pore leading to inhibition of pollen germination. In some families such as Poaceae, the rejection reaction is completed within few minutes of pollination and subsequent rejection or acceptance occurs within 10 minutes. In most of the cases, the recognition/rejection reaction occurs after the pollen tube has grown to about two-third length of the style. The proteins incorporated during the pollen wall development in the intine contribute to incompatibility. In most of the GSI systems, the rejection reaction is delayed and synthesis or activation of recognition substances is also delayed.

SAQ 5

State whether the statements are 'True' or 'False' :

- i) Incompatibility is not a genetically controlled process.
- ii) Self-incompatibility response is genetically controlled by one or more multi-allelic loci.
- iii) According to 'Opposition S- alleles' hypothesis a single gene 'S' with several alleles controls the incompatibility reactions.

- iv) The distyly condition is controlled by two genes.
- v) Two genes control tristyly.
- vi) The dry stigma possesses a hydrated layer called pellicle over the cuticle which consists of lipids and proteins.
- vii) In gametophytic incompatibility, the callose deposition occurs on the stigma.
- viii) Taxa having dry stigma are associated with sporophytic incompatibility while those having wet stigma are associated with gametophytic incompatibility.

11.7.1 Factors Involved in Incompatibility

You have studied about genetic basis of incompatibility but there are other factors also involved which we are going to discuss.

Pollen wall proteins

The pollen wall proteins have different origins. The exine held proteins are of sporophytic origin while the intine held proteins are of gametophytic origin. According to Heslop - Harrison, the sporophytic incompatibility is due to proteins found in exine while the gametophytic incompatibility is due to proteins found in intine. In case where the pollination is incompatible, the rejection reaction starts when pollen fails to germinate or produces a short germ tube. The growth of pollen tube is restricted. It soon gets closed because of callose deposition. The callose plug is formed between the plasma membrane and pectocellulosic layer of the stigmatic papilla after pollination. The main components involved in the rejection are supposed to be present in the exine i.e. exine held proteins. This confirmed that the component involved in the rejection reaction is sporophytic in nature.

Stigma surface proteins

The proteins present on the stigma surface are supposed to be involved in the pollen recognition rejection process particularly in the intraspecific crosses. It has been noted that water soluble substances released from the stigma inhibit pollen germination.

In members of family Brassicaceae, having stigma covered with cuticle. The pollen which penetrates the cuticle is called compatible pollen whereas if pollen that fails to grow through the cuticular layer it is referred as incompatible pollen. The compatible stigma activates the enzyme cutinase which is responsible for pollen tube penetrating the cuticle. It has been suggested that stigma surface inhibition leading to incompatible pollen is based on the following features:

- 1) The rejection-reaction exhibited by incompatible stigma shows lack of copious exudates i.e. stigma remains dry. These stigmas possess an extremely active, hydrated pellicle over the cuticle.

- 2) The pellicle comprises of lipids and a variety of proteins which possess esterase activity.
- 3) The pollen when present on the stigma stimulates exudation of water by papillate cells. The hydrated condition of stigma stimulates the outflow of exine bound proteins.
- 4) The released exine proteins are received by the pellicle. The exine and pellicle proteins interact with each other resulting in an incompatible reaction.
- 5) The formation of callose plugs is another feature that leads to rejection reaction.

Besides these, the stylar region also shows rejection reaction. In gametophytic self- incompatibility the pollen grains germinate, the pollen tube grows and reaches the style and then the rejection reaction sets in stopping further growth of the pollen tube. Inhibition in stylar region occurs because:

- the cells of the style secrete S allele specific proteins which induce incompatibility reaction
- the degeneration of the tube cytoplasm and thickening of pollen tube results in incompatibility.
- the style show high RNAase activity. The entry of RNAase in the pollen tube restricts its growth.
- the tip of the pollen tube in an incompatible style show different structure and is unable to absorb the secretions from the pistil ,resulting in retarded growth.
- the pollen tube in an incompatible style show abnormal structures such as branching of the tip of the pollen tube, development of callose plugs or restricted growth of pollen tube.

11.8 METHODS TO OVERCOME INCOMPATIBILITY

The incompatibility arises because of several factors; hence various methods need to be followed to remove it. Some of the methods have been listed below.

In plants with sporophytic incompatibility stigma has a cuticle layer which acts as the incompatibility barrier. Cutinase enzyme removes the layer of the cuticle to allow the reaction to take place. It has been considered as one of the important recognition sites. Treatment with Ca supported pollen tube growth in plants with incompatible style. Example- *Oenothera*. In *Petunia* emasculation of immature flowers reduced pistil elongation and weakened incompatibility barrier.

Various modifications of pollination methods can be tried to remove incompatibility. These include:

Bud Pollination: The self-incompatibility in the bud stage can be overcome two to seven days before anthesis. In *Petunia axillares* inhibition is removed if the buds are self-pollinated two days before anthesis. At the bud stage the stigma lacks exudates, which appear only during anthesis. Thus, if the stigma is self-pollinated at bud stage when the factor responsible for the exudates has not yet appeared, the pollen tubes will grow normally and effect fertilization. This method is successful in both the gametophytic and sporophytic systems.

Mixed pollination or Use of mentor pollen: The stigma can be pollinated by a mixture of incompatible and compatible pollens. The compatible pollen is known as mentor or recognition pollen. When the mentor pollen (compatible pollen) and incompatible pollen are mixed, the recognition protein from the compatible pollen masks the inhibition reaction and allows incompatible pollen to germinate. The mentor pollen also provides growth promoting or regulating substances to the pollen and hence the incompatible pollen attains growth.

Mentor pollen has been used successfully to overcome incompatibility by using them along with live incompatible pollen. The compatible pollen is made ineffective by irradiation or repeated freezing and thawing or treating with chemicals like ethanol for fertilization. For example- in *Cosmos* mentor pollen was found effective in overcoming self- incompatibility. Mentor pollen has been successfully used in species of *Brassica oleracea*, *Petunia*, *Nicotiana* and *Lilium*. The function of mentor pollen has been suggested as providing recognition substances to incompatible pollen or to provide pollen growth substances to incompatible pollen.

Intraovarian pollination: Incompatibility occurring on stigma or in the style can be overcome by this type of pollination. Intraovarian pollination occurs through a slit or pore. Ovary is sterilized with ethanol and two holes are made inside the ovary. One hole is used for injecting pollen and the other hole is used for ejecting air out. The holes are sealed with petroleum jelly and the pollen is allowed to germinate inside the ovary. The method has also been successful in members of Papaveraceae like *Papaver rhoeas* and *P. somniferum*.

Stub pollination: In Stub pollination stigma is removed and pollen is placed directly on style. Those incompatibilities that are restricted to the stigma or to the length of the style can be overcome by removing the stigma and part of the style. The stigmatic surface of *Ipomoea trichocarpa* is the primary site of incompatibility and if the stigmatic lobe is removed and the cut surface pollinated then the pollen tube grows uninhibited in to the ovule. In *Nicotiana tabacum* the style is longer than the other species, as a consequence the pollen tube fails to reach the ovule. The removal of a large part of the style from *N. tabacum* made the crossing successful.

In vitro pollination: This method was developed to overcome prezygotic barriers to fertility. The exposed ovules were directly dusted with pollen grains and then cultured in a suitable nutrient medium that supported both the germination of pollen as well as the development of fertilized ovules. The technique was developed by Kanta (1962) in *Papaver somniferum*. Better results were obtained by culturing the ovules within the intact placental tissue as such the technique is also termed as placental pollination.

Removal of stigma

Removing of stigma or upper part of the style can also help in removing the inhibition factor that lies on the stigma. Sometimes the length of the style also hinders the process. Removal of the stigmatic lobe or part of the style helped in overcoming self- incompatibility by facilitating pollination in *Ipomoea*.

Modification of stigmatic surface

Application of lecithin, concanavalin A to stigma helps in overcoming incompatibility in *Brassica*. Treatment of stigma with detergent Triton -X or organic solvents such as hexane also proves useful in overcoming incompatibility. Studies also showed that pre treatment of stigma with NaCl blocks incompatibility reaction in *Brassica campestris*.

Heat treatment

High temperature has also been found to inactivate the incompatibility barrier. Heating of style in water at 50°C for 6 minutes before pollination has shown to remove incompatibility factor. In *Lilium*, self- incompatibility can be overcome by giving an interactive treatment of high temperature exposure and 1% naphthaleneacetamide in lanolin. In rye, temperature of 30°C proved effective in inhibiting incompatibility.

Irradiation

Treatment with gamma rays proved effective in removing incompatibility barrier in *Lycopersicon peruvianum*. A high seed set was achieved in the plant due to prevention of flower abscission at early stage.

Chemical treatment

In cases where abscission of flower is the barrier to compatibility, application of chemicals such as 2, 4 Dichlorophenoxyacetic acid, to pedicel proved effective in increasing life span of flower and *Ipomoea batatas*. Application of p-chloromercuribenzoate and kinetin proved useful in *Lilium longifolium* and *Oenothera oraganensis*.

Increased CO₂ level

Raising of atmospheric level of CO₂ by 100 fold proved useful in overcoming self- incompatibility in *Brassica*.

Parasexual hybridization

The hybridization of the somatic cells has helped to overcoming sexual incompatibility. The process involved isolation of protoplasts, their fusion and culture of the hybrid protoplast to generate plants. The fusion of isolated protoplasts has proved successful in getting rid of incompatibility.

11.8.1 Significance of Incompatibility

Incompatibility determines the degree of inbreeding and outbreeding of plants. Self- incompatibility is a natural way to circumvent extensive selfing that promotes homozygous individuals and eventually causes inbreeding depression (accumulation of lethal recessive genes). The prevention of inbreeding results in high genetic diversity. Genetic diversity is important for maintaining the health and survival of species. The process also leads to the production of hybrids.

SAQ 6

- a) Incompatibility is important for plants. Why?
- b) Answer in one word :
- an outer exine wall is composed of the chemically resistant polymer called
 - The pollen tube wall is composed of
 - In plants with dry stigma, this enzyme present in the pollen tube digests the stigmatic cuticle.
 - The process of fusion of sperm (male gamete) with the egg to form zygote is called
 - The process in which fusion of two gametic nuclei is hindered due to some defect.
-

11.9 SUMMARY

- The pistil develops mechanisms to recognize pollen and permit growth of pollen tubes through style and stigma. Stigma provides conditions for the retention and germination of pollen grains.
- In plants having dry stigma, the enzyme cutinase present in the pollen tube digests the stigmatic cuticle. The tube penetrates the pectocellulosic wall of the papillae and traverses the intercellular spaces of the stigma and style. Finally it passes through the intercellular matrix or cell walls of the transmitting tissue. In plants with dry stigma and hollow style, the cuticle over the papillae is continuous and extends throughout the stylar canal. In plants with wet stigma and closed style, the pollen tube enters the intercellular matrix of the stigma before entering the style.
- The factors such as high temperature and humidity, sugars and elements such as boron and calcium facilitate the germination of the pollen tube. Enzymes such as cellulase, pectinase and callase present in pollen grains increase the rate of pollen tube elongation. The hormones such as auxins and gibberellins also promote pollen tube growth.
- The pollen tube grows through the micropyle and enters one of the two synergids through filiform apparatus. In many species, one of the two synergid degenerates before the entry of the pollen tube. In this case the pollen tube enters through the degenerated synergid. In other plants, both synergids remain intact at the time of pollen tube entry. The synergid that receives the pollen tube starts degenerating.
- One of the sperm gets connected to the vegetative nucleus by a cytoplasmic extension, thus forming a three - celled male germ unit

(MGU). The organisation of the cells in the MGU is fixed and determines which sperm will fuse with the egg, and which one will participate in triple fusion.

- After traversing the stylar region, pollen tube reaches the female gametophyte. The sperms are released and fertilization takes place. Double fertilization is an important feature unique to angiosperms. This involves fusion of one sperm or the male gamete with the egg to form zygote (progenitor of next generation). The process is called syngamy. The other sperm fuses with the secondary nucleus or the polar nuclei resulting in triple fusion (one haploid male gamete and two haploid polar nuclei = $3n$), the primary endosperm nucleus.
- The gametophyte (pollen) and the sporophyte (pistil) interaction leads to recognition i.e. acceptance or rejection of the mating partners. The female gamete is located in the embryo sac inside ovule present in the ovary in angiosperms. The pollen grains carry male gametes through pollen tube that travels from stigma to ovary. The pollen tubes traverse stigma and style region to reach the ovary. Sometimes the fertile pollen fails to accomplish fertilization process and fertilization does not take place to form a viable embryo. The process is referred as sexual incompatibility. Incompatibility can occur between the individual of same species-intraspecific or of different species-interspecific.
- Self- incompatibility (SI) is a genetically controlled pollen-pistil recognition system. The incompatibility phenotype of the pollen is determined by two genetically distinct forms of SI: gametophytic SI (GSI) and sporophytic SI (SSI). GSI appears to be the most common mode of self- incompatibility. Self- incompatibility in flowers of the same morphological character is genetically controlled by S-locus. The SI system is controlled by a single Mendelian locus (the *S*-locus), which is comprised of a number of tightly linked genes, coding for pollen–pistil recognition, which function as a single locus. The *S*-locus is comprised of a gene complex that encodes specific proteins involved in recognition and rejection of ‘self’ pollen.
- The incompatibility arises because of several factors. Various methods need to be followed to remove it. These include bud pollination, mixed pollination, in vitro pollination, test tube pollination, stub pollination, removal of stigma, modification of stigmatic surface, chemical treatment, heat treatment, irradiation etc.

11.10 TERMINAL QUESTIONS

1. Why is hydration important for pollen germination?
2. What are the factors that regulate the adhesion of the pollen grains on the stigmatic surface?
3. Differentiate between recognition and rejection reaction.
4. Different barriers prevent fertilization. Discuss

5. Differentiate between:
 - i) gametophytic and sporophytic incompatibility
 - ii) homomorphic and heteromorphic self- incompatibility
6. What is double fertilization? Describe with suitable diagram.
7. Describe various methods to curb incompatibility.

11.11 ANSWERS

Self-Assessment Questions

1.
 - a)
 - i) microspores
 - ii) primexine matrix
 - iii) volatile
 - iv) germination
 - v) exine
 - vi) pollen coat
 - b)
 - i) exine
 - ii) hydration
 - iii) stigmatic exudates
 - iv) callose
 - v) cutinase
 - vi) intercellular spaces
 - vii) auxins and gibberellins
 - c) Refer to Section 11.2
 - d) Refer to Section 11.2
2.
 - a)

i) True;	ii) True;	iii) False;
iv) False;	v) True;	vi) True.
 - b)
 - i) esterase (cutinase)
 - ii) chalazogamy
 - iii) filiform
 - iv) synergids
 - v) chemotropic
 - vi) male germ unit (MGU)
 - c) Refer to Subsection 11.3.3.

3.
 - a) Refer to Subsection 11.3.3.
 - b) Refer to Subsection 11.3.3.
 - c) Refer to Section 11.4.
4.
 - a)
 - i) intraspecific incompatibility
 - iii) homomorphic type of self- incompatibility
 - iii) unilateral incompatibility
 - ii) interspecific incompatibility
5.

i) False;	ii) True;	iii) True;	iv) False;
v) True;	vi) True;	vii) False;	viii) True
6.
 - a) Incompatibility irrespective inter or intraspecific determines the degree of inbreeding and outbreeding of plants. Self-incompatibility is a natural way to overcome or avoid selfing that promotes homozygous individuals and results in accumulation of lethal recessive genes via inbreeding. Interspecific incompatibility prevents hybridization.
 - b)
 - i) sporopollenin
 - ii) polysaccharides
 - iii) cutinase
 - iv) syngamy
 - v) sexual incompatibility

Terminal Questions

1. Hydration of the pollen triggers the release of pollen wall proteins which govern the compatibility and interaction between two parents.
2. Stickiness of the pollen, stigma, exine pattern, composition of the pellicle, surface exudates, electrostatic forces and specificity between two parents are some of the factors that regulate the adhesion of the pollen grains on the stigmatic surface.
3. **Recognition reaction**

The compatibility has been decided at the molecular level by the pistil. In SSI systems, the acceptance or rejection of the pollen is decided by the stigmatic surface. In GSI systems this happens mostly in style.

Rejection reaction

The physiological and biochemical processes are set in the pistil by the recognition reaction specific to the type of pollen that land on the stigma. Inhibition can occur on stigma itself thus preventing the germination of pollen or its entry into style. In GSI system, it occurs in style leading to inhibition of pollen tube growth or its bursting.

4. Refer to Section 11.5.
5.
 - i) Refer to Subsection 11.6.2.
 - ii) Refer to Subsection 11.6.2.
6. After traversing the stylar region, pollen tube reaches the female gametophyte. The sperms are released and fertilization takes place. Double fertilization involves fusion of one sperm or the male gamete with the egg to form zygote (progenitor of next generation). The process is called syngamy. The other sperm fuses with the secondary nucleus or the polar nuclei resulting in triple fusion (one haploid male gamete and two haploid polar nuclei = $3n$), the primary endosperm nucleus.
7. Refer to Section 11.8.



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