Pollination, Fertilization and Seed Development

26

Rashmi Shakya and Satish C Bhatla

Life cycle of plants is fundamentally different from that of animals. It is characterized by the presence of two distinct multicellular generations, referred as sporophytic (diploid) and gametophytic (haploid) generation which alternate with each other during the life cycle. Male and female reproductive organs in plants are stamens (androecium) and carpels (gynoecium), respectively. Both the reproductive structures produce haploid spores as a result of meiosis, namely, microspores (male) and megaspores (female). These spores undergo repeated mitotic divisions to produce male and female gametophytes, called as microgametophyte and megagametophyte, respectively. Development of male gametophyte takes place inside the anther, whereas female gametophyte develops inside the ovule. Upon maturity male and female gametophytes divide mitotically to produce male and female gametes, i.e., sperm and egg, which fuse to form zygote that develops to give rise to sporophytic plant (Fig. 26.1).

This chapter begins with description of male and female gametophyte. Later, transfer of pollen, followed by detailed account of events associated with pollen-pistil interaction, leading to double fertilization. Genetic basis of sexual incompatibility barrier has then been discussed describing three mechanisms worked out till date. At last, development of seed has been explained as a complex, coordinated process, including embryogenesis, endosperm development, and maturation of seed, conferring it to desiccation tolerance capability.

26.1 Development of Male Gametophyte

Pollen grain is the male gametophyte in flowering plants. Development of pollen grains occurs inside the anther which is the fertile portion of the stamen. Typically, an anther is composed of a well-defined anther wall which encloses a mass of sporogenous tissue inside the locules. The wall of anther is differentiated into four regions which are centripetally organized as epidermis (single layer), endothecium

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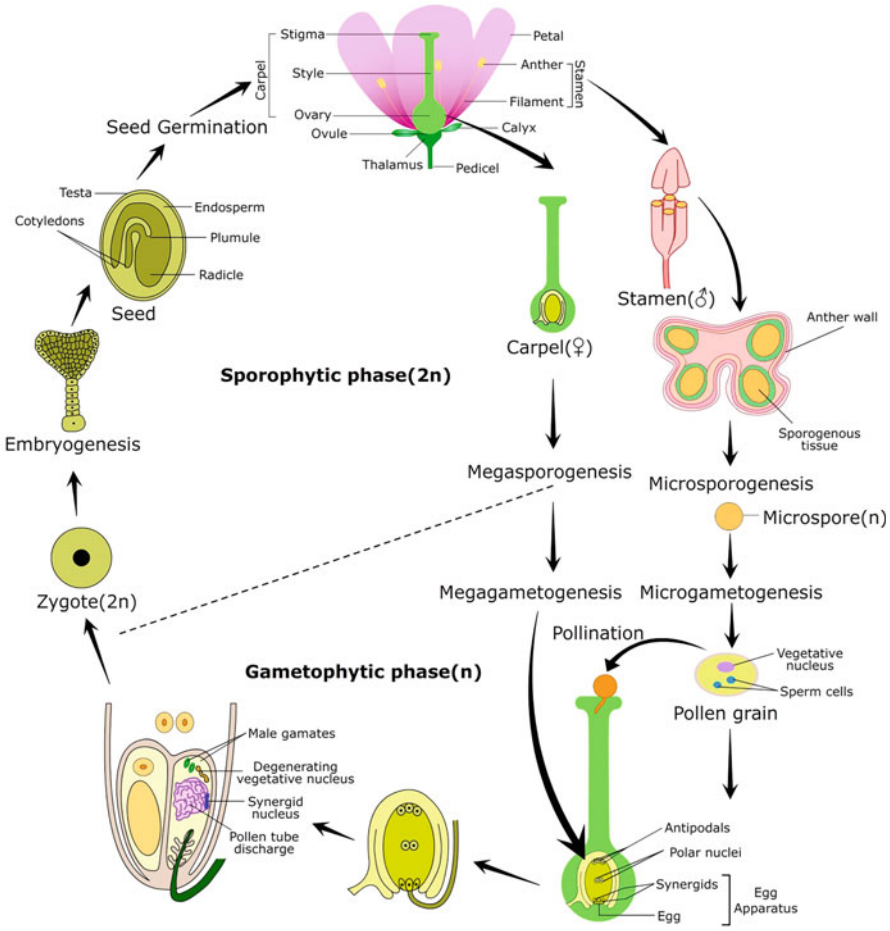


Fig. 26.1 Alternation of sporophytic and gametophytic phases in the life cycle of flowering plants

(single layer), middle layers (2–3 layers), and tapetum (Fig. 26.2; Table 26.1). **Tapetum** consists of secretory cells which completely surround the inner sporogenous tissue. Sporogenous tissue inside the anther locule differentiates into microsporocytes, i.e., **pollen mother cells (PMCs)**, which undergo meiosis to form microspores. Microsporocytes undergo meiosis to give rise to haploid microspores which are joined to each other as tetrads through special cell wall (SCW), primarily made up of callose (Fig. 26.3). Secretory cells of the tapetal layer release callase and other cell wall-degrading enzymes which lead to the hydrolysis of SCW, thus separating the microspore tetrads into individual microspores. Each microspore develops into a **pollen grain**. During the development of pollen grains, the cytoplasm of microspores becomes highly vacuolated, and nucleus migrates to one side of the cell wall, conferring polarity to microspores. The

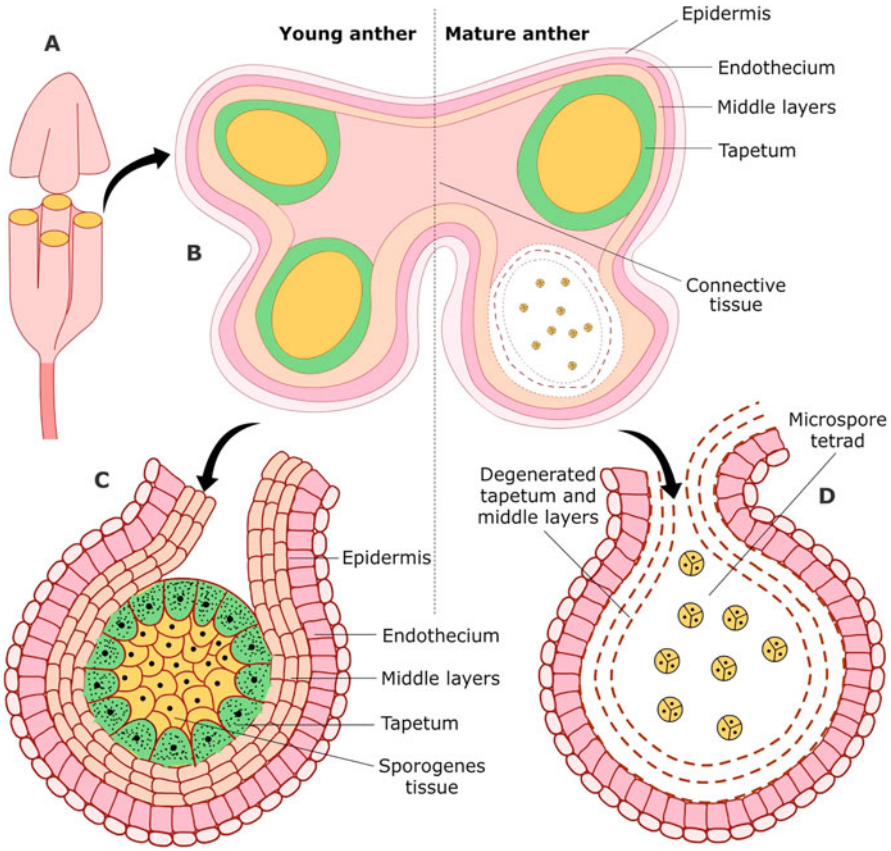


Fig. 26.2 Transverse section of young (**a**) and mature anther (**b**) showing sporogenous tissue and microspore tetrads, respectively

Table 26.1 Structure of stamen and carpel

Stamen	Carpel
Filament	Stigma
Anther	Style
Anther wall	Ovary
Epidermis (single layered)	Ovary wall
Endothecium (single layered)	Placenta
Middle layers (2–3)	Ovule(s)
Tapetum (single layered)	Funiculus
Microspore mother cell	Integuments and micropyle
(microsporocyte)	Nucellus
	Megaspore mother cell
	(megasporocyte)

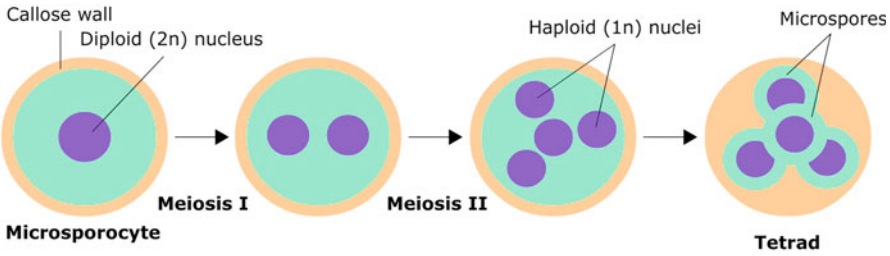


Fig. 26.3 Microsporogenesis: formation of four haploid microspores as a result of meiosis of microsporocyte (pollen mother cell)

polarized microspore then divides asymmetrically by pollen mitosis I (PM I) to give rise to a large vegetative cell (or tube cell) and a small generative cell (or male germ cell). The division of the cytoplasm is unequal so that most of the cytoplasmic organelles, which includes mitochondria and plastids, remain in the vegetative cell. The nucleus of generative cell possesses highly condensed chromatin as compared to the vegetative nucleus. Initially, generative cell is attached to the microspore cell wall, and formation of hemispherical callose layer takes place between the plasma membranes separating generative and the vegetative cells (Fig. 26.4). Eventually, generative cell detaches itself from the microspore cell wall and gets harbored inside the cytoplasm of the vegetative cell ("cell within a cell"). Subsequently, the spherical generative cell assumes an elongate crescent shape which has been implicated in its easy entry inside the growing pollen tube. The crescent shape of the mature generative cell is retained by the sperm cells it produces. Microtubules present in the cytoskeleton of the generative cell are responsible for maintaining this shape. The shape of the generative cell is gradually lost upon isolation of generative cell from the pollen. During maturation of pollen grains, accumulation of carbohydrates and lipids takes place to support the upcoming pollen germination and pollen tube formation. Depending on the species, the tapetal layer may either remain intact at the periphery or may become amoeboid and migrate into the locule during pollen development. In both the cases, tapetum performs secretory function and provides nutrition to the developing pollen grains. Eventually, tapetal cells undergo programmed cell death (PCD), releasing their contents inside the anther locule. Tapetal cells perform significant role in the supply of nutrients, enzymes, and cell wall precursors to the developing pollen grains. For this reason, any defect in tapetum causes abnormal development of pollen grains, and consequent defects in their fertility. Mostly, anther dehiscence takes place at this stage when the pollen grains are two celled. Generative cell undergoes pollen mitosis II (PM II), and the timing of PM II varies from species to species. Mostly, generative cell undergoes PM II division to form two sperm cells when the pollen grains are still inside the anther locule. However, the generative cells may sometimes undergo PM II to form two male gametes inside each pollen grain after it has alighted onto stigma surface (e.g., *Holoptelea integrifolia*). PM II may take place after germination of pollen grains on stigma surface (e.g., *Zea mays* and *Nicotiana tabacum*). PM II in the generative cell

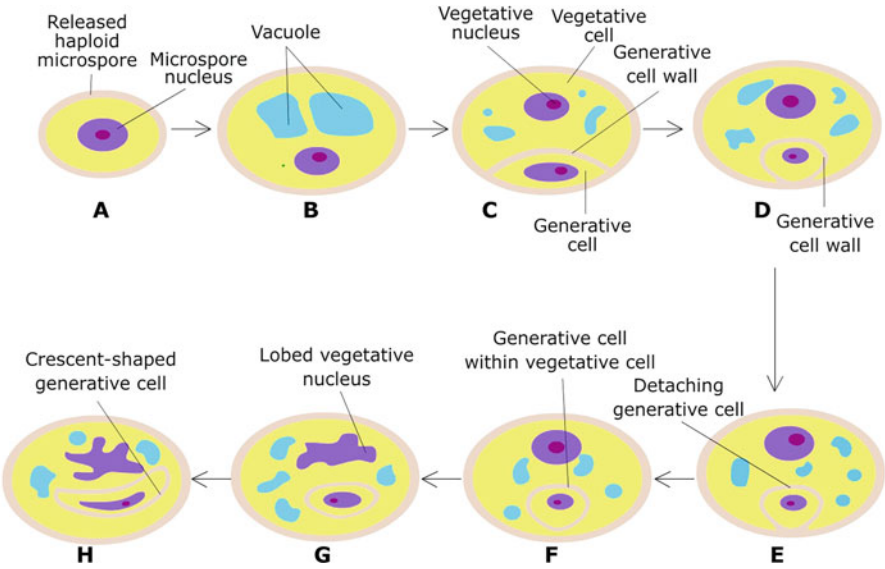


Fig. 26.4 Microgametogenesis: formation of generative cell and vegetative cell due to mitotic division of the microspore

may take place in the pollen tube before it reaches the embryo sac (most common condition). Rarely, generative cell undergoes PM II after the pollen tube has reached the embryo sac (e.g., in *Euphorbia terracina*). The chromatin in the nucleus of sperm cells is highly condensed. Since generative cells are devoid of mitochondria and chloroplasts, the sperm cells also lack these organelles. Therefore, these organelles are inherited maternally in almost 90% of angiosperms.

Upon attaining maturity, the cell wall of pollen grains shows a great deal of variation in architecture which is ecologically important in the pollination process. Pollen wall is highly complex, and it mainly includes three domains which differ not only in their structure and chemical composition but also in biological and physiological significance. The three domains are exine, intine and pollen coat (tryphine) (Fig. 26.5). The process of cell wall formation in pollen is initiated immediately after meiosis. Deposition of ephemeral callose wall, i.e., SCW, is followed by the layers of sexine (ectexine), nexine (endexine), and finally the intine. Sporopollenin is a chemically inert biological polymer and has been suggested to consist of covalently linked phenolic and fatty acid-derived constituents. Initially sporopollenin precursors synthesized and secreted by the microspores contribute to the formation of exine layer. However, after dissolution of SCW, sporopollenin precursors are mostly provided by the secretory cells of the tapetal layer. The intine layer is primarily made up of cellulose and pectins. Mostly, pollen wall possesses elongated areas called as apertures or the germ pore region where exine is either missing or very thin. Through these germ pores, pollen tube emerges during pollen germination on the surface of stigma. In grasses, however, a pectin rich layer, known as Z-layer,

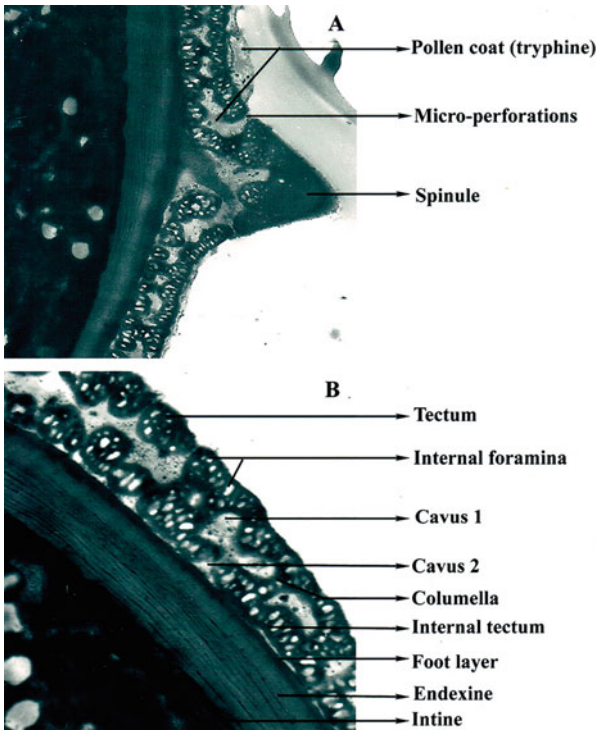


Fig. 26.5 Transmission electron micrograph (TEM) of pollen wall of sunflower showing (**a**) three domains, intine, exine, and pollen coat (4600X) and (**b**) details of inter-spinular region (8400X)

is present in between exine and intine. This layer is quite thick at germ pore areas and is referred to as "Zwischenkorper." Pollen wall diversity is often a characteristic feature of each species and is significant in assigning taxonomic position as well. In insect or self-pollinated species, like *Brassica*, *Arabidopsis* (Brassicaceae), and *Felicia* (Asteraceae), the pollen coat is thick. A large amount of pollen coat substances is present on the outer surface and interbacular cavities of ectexine. In wind- or cross-pollinated species, such as maize, the pollen coat is thin. Basically, two types of pollen coat materials are produced by tapetum layer of anther, namely **pollenkitt** and **tryphine**. Pollenkitt is the sticky substance present around most of the pollen grains pollinated by animals, whereas tryphine is exclusive to members of Brassicaceae which are entomophilous. The degeneration of tapetal cells at microspore stage of male gametophyte development leads to the formation of tryphine at the pollen surface. On the other hand, degeneration of tapetal layer at later stages causes pollenkitt deposition on the pollen wall. The nature of pollen coat substances is quite variable in different species. Pollen coat substances, mainly pollen coat proteins (PCPs) and lipids, have been implicated in several functions starting from holding the pollen before anthesis, at the time of pollination, and during initial stages of pollen-stigma interaction. PCP-B class of coat proteins have recently been reported to play a significant role in the hydration of pollen on the stigma papillae.

26.2 Development of Female Gametophyte

Carpel, the female reproductive structure in flowering plants, consists of stigma, style, and ovary. Ovules which contain the female gametophyte (embryo sac) are present inside the ovary. Ovule primordia arise along the placenta as projected rounded tips. During the early stage of ovule development, three regions can be identified. The basal proximal region gives rise to funiculus. The distal or micropylar region at the tip produces the nucellus. The central region, chalaza, gives rise to outer layers of the ovule called as **integuments**. The megaspore mother cell is characterized by its large size and large nucleus, and its dense cytoplasm gets differentiated in the nucellar tissue (Fig. 26.6a). The development of embryo sac is quite complex and diverse as compared to pollen. Approximately 15 types of embryo sac development patterns have been reported among angiosperm species. The most common pattern of embryo sac development observed in *Polygonum* and is known as ***Polygonum* type** of embryo sac development. The megaspore mother cell undergoes meiosis to form four megaspores out of which the three positioned toward the micropylar end degenerate (Fig. 26.6b–d). The functional megaspore divides three times by free nuclear division giving rise to a multinucleate cell called as **syncytium** (Fig. 26.6e–h). Of the eight nuclei present in the immature embryo sac, four move toward the chalazal end and the remaining four toward the micropylar end. Three nuclei, at the micropylar and chalazal ends, undergo cellularization process. One nucleus from each pole migrates toward the center. These are called as **polar nuclei**. Plasma membrane develops around both the polar nuclei and surrounding cytoplasm. Thus, mature "*Polygonum* type" of embryo sac contains

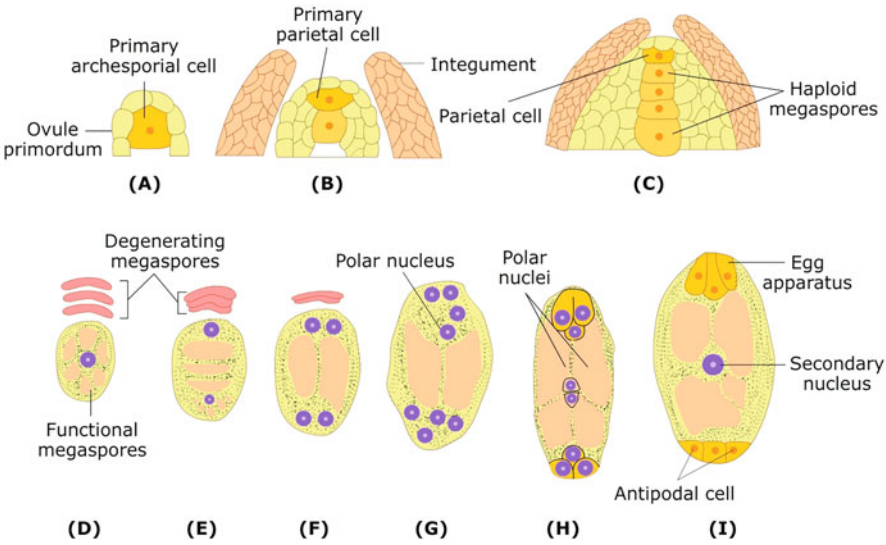
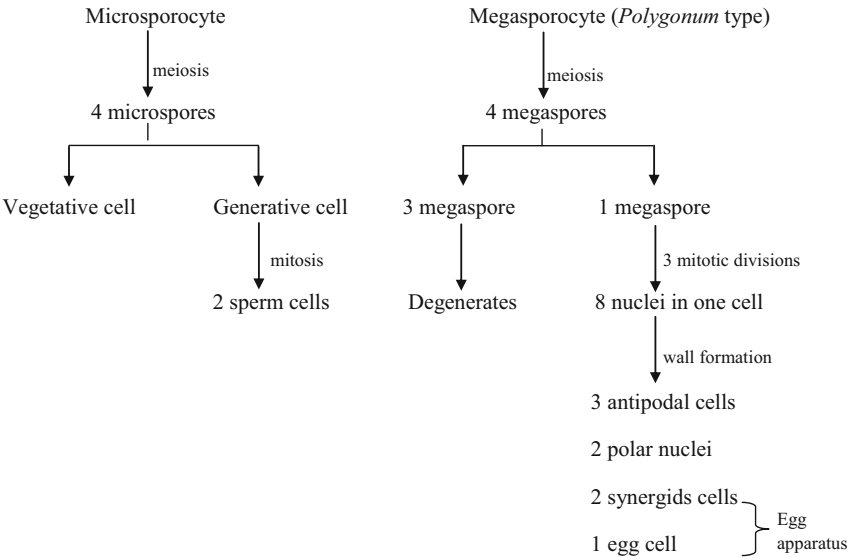


Fig. 26.6 (**a–d**) Development of megaspore, i.e., megasporogenesis, and (**e–i**) development of megagametophyte, i.e., megagametogenesis to form embryo sac

Table 26.2 Development of male and female gametophyte

Male gametophyte (microgametophyte)	Female gametophyte (megagametophyte)
	

eight nuclei and seven cells (Fig. 26.6i). The three cells toward the micropylar end organize themselves into an **egg apparatus** with one **egg** embraced by two **synergids**. The characteristic feature of the egg apparatus is the presence of **filiform apparatus** at the micropylar tip in the synergids. The three cells toward chalazal end are called as **antipodal cells**. The large, binucleate cell in the center containing two polar nuclei is called as **central cell**. The central cell is also regarded as a gamete since one of the male gametes fuses with the central cell to form triploid **primary endosperm nucleus (PEN)**. However, the developmental fate of PEN is quite different from the fusion product of another male gamete and egg, i.e., **zygote**. PEN develops to form endosperm, and the ploidy of endosperm is dependent on the type of embryo sac. Table 26.2 summarizes the development of male and female gametophyte in flowering plants.

26.3 Pollination and Double Fertilization

Transfer of pollen grains to the stigma surface is a prerequisite to ensure successful development of fruit and seed set in flowering plants. This process of pollen transfer from anthers to the stigma surface is known as **pollination**. For the success of

pollination, it is imperative that viability of pollen and period of stigma receptivity must coincide. Pollen may be transferred onto the stigma surface on the same flower (**self-pollination or autogamy**) or to the stigma of another flower present on the same plant (**geitonogamy**) or on a different plant (**xenogamy**). The latter two processes constitute **cross-pollination** whose success is dependent on the intervention of several external agencies and various factors, such as temperature. Pollen grains in certain plants, such as tomato, get damaged by heat, whereas some others can tolerate high temperature. After pollen has alighted onto the surface of stigma, a number of structural, biochemical, and physiological events occur as a part of cellular dialogue between pollen and pistil. Successful completion of these events leads to a complex phenomenon known as **double fertilization,** which is characterized by the fusion of one sperm nucleus with the egg cell to form zygote, and the second sperm nucleus fuses with polar nuclei or secondary nucleus to form PEN. Development of zygote results in the formation of embryo (embryogenesis), whereas PEN develops to form endosperm, the nutritive tissue that provides nourishment to the developing embryo.

26.3.1 Pollen Adhesion and Hydration

The nature of pollen coat and stigma surface determines the possibility of contact between these two surfaces. Stigma surface may be termed as dry or wet type depending on the presence of stigmatic exudates during the receptive period. In "wet-type" **stigmas**, the surface is characterized by exudates which are a complex mixture of polysaccharides, proteins, and lipids. In "dry-type" **stigmas**, papillae are covered by a proteinaceous pellicle, cuticle, and cell wall (Table 26.3). The surface in wet type of stigma, such as in *Nicotiana* and *Lilium*, is unable to discriminate between compatible and incompatible pollen grains. Thus, pollen from any species falling onto such stigma surface gets adhered to the exudates secreted during the receptive phase. However, in plants possessing dry-type stigma, such as *Arabidopsis*, pollen adhesion is a highly species-specific process. In *Arabidopsis*, self-pollen grains adhere more tightly to the surface of stigma as compared to the pollen from other unrelated plants, such as *Petunia hybrida* or other members of Brassicaceae, demonstrating a clear species preference during pollen adhesion. Upon landing of compatible pollen on the papillae of dry stigma, mobilization of pollen coat substances takes place on the papillae. This results in the formation of an interface (popularly referred to as "attachment foot") between pollen and stigmatic papillae where interaction between molecules from these two domains occurs.

Table 26.3 Type of stigma surface and their chemical nature

Type	Chemical constituents	Example
Dry type	Proteinaceous pellicle and cuticle	*Arabidopsis*, sunflower, *Brassica*, *Senecio*
Wet type	Mixture of polysaccharides proteins and lipids	*Lilium*, *Petunia*, *Nicotiana*, *Papaver*

Adhesion of pollen to stigmatic papillae is dependent on the biophysical and biochemical interactions between pollen coat substances and stigma surface proteins. Removal of pollen coat with organic solvents, such as cyclohexane, acetone, and diethyl ether, adversely affects pollen adhesion and its hydration. It has been reported that the strength of compatible pollination declines upon removal of the pollen coat and stigma waxes. Like pollen adhesion, hydration of pollen on wet stigma surface is not regulated, whereas it is highly regulated in dry stigma. In species possessing dry type of stigma, such as in *Arabidopsis* and sunflower (*Helianthus annuus*), exine components mediate the adhesion of pollen to the stigmatic papillae. This adhesion is highly species specific as the strength of adhesion is much stronger in intraspecific pollination as compared to interspecific pollination. It is thought that lipids and proteins present at the interface enable changes in the properties of the underlying papillae which lead to the transport of water and ions from the stigmatic papillae resulting in hydration of pollen. Two mechanisms have been implicated in pollen hydration. First, movement of water molecules through aquaporin channels located in the plasma membrane of papillae may take place. Second, targeted vesicular exocytosis to the papillae surface upon arrival of compatible pollen grain also facilitates pollen hydration among the members of Brassicaceae. Experiments have shown that defect in a gene required for normal exocytosis of Golgi vesicles leads to failure of pollen hydration on stigma surface. In species possessing wet type of stigma, such as *Lilium* and *Petunia*, pollen hydration is not a very specific requirement since water present in exudates supports hydration of pollen. In addition, stigmatic exudates contain lipids, proteins, sugars, and flavonoids that support pollen germination as well. It is believed that lipids present in the exudates substitute for the function of pollen coat and are involved in directional growth of pollen tube. Mutants lacking these exudates are female sterile and can be rescued by exogenous application of lipids on stigma surface.

26.3.2 Ca^{2+} Triggered Polarization of Pollen Grain Before Emergence of Pollen Tube

Pollen grains land on the surface of stigma in a highly desiccated state and are inactive, i.e., they cannot germinate. Hydration of pollen on stigma surface renders it physiologically active. Ca^{2+} plays a very crucial role in pollen germination and subsequent growth of pollen tube. Influx of Ca^{2+} into the vegetative cell sets up reorganization of cytoskeleton which leads to polarization of pollen. Immediately following hydration, concentration of free cytosolic Ca^{2+} (Ca^{2+}_{cyt}) increases below the germ pore region from where the emergence of pollen tube takes place. The concentration of [Ca^{2+}_{cyt}] remains high until the pollen tube emergence. Accumulation of both actin microfilaments and secretory vesicles takes place below the germ pore area. Migration of the vegetative nucleus takes place in such a manner to facilitate the movement of sperm cells ahead of vegetative cell in the pollen tube during germination. Table 26.4 outlines the initial events prior to pollen tube emergence during pollen stigma interaction.

Table 26.4 Initial events (prior to pollen tube emergence) during pollen-stigma interaction

S. No.	Events during pollen-stigma interaction
I.	Adhesion
	Formation of attachment foot
	Molecular interactions of proteins and lipids
	Changes in permeability patterns due to opening of aquaporin channels on stigmatic papillae
II.	Hydration of pollen
	Caused by targeted vesicular exocytosis to the papillae
III.	Ca^{2+} influx into the vegetative cell
IV.	Accumulation of actin and vesicles below the germ pore area

Table 26.5 Rate of growth of some tip-growing structures in plants

Structure	Rate of growth	Examples
Pollen tube	2.8 μm s^{-1}	Maize
	0.2–0.3 μm s^{-1}	Lily
	55 μm s^{-1}	*Conospermum* species
Root hair	0.01–0.04 μm s^{-1}	*Arabidopsis*
Chloronema tip cell	0.001625 μm s^{-1} (5.85 μm h^{-1})	*Physcomitrella patens*
		Funaria
Fungal hyphae	0.201 μm s^{-1}	*Neurospora crassa*
	0.038 μm s^{-1}	*Rhizoctonia solani*

26.3.3 Apical Growth of Pollen Tube Tip and Its Regulation

Pollen tube elongates by tip growth following germination process. The growth rate of pollen tube is quite rapid (more than 5 μms^{-1}) as compared to that of root hair (10–40 nms^{-1}) which also grows by tip growth. In case of maize, pollen tubes attain length up to 40 cm. Table 26.5 summarizes the rate of growth in some tip-growing structures in plants. The growth of the pollen tube ceases at the surface of stigmatic papillae when rejection of pollen takes place in species exhibiting sporophytic self-incompatibility (SSI). However, in species showing gametophytic self-incompatibility (GSI), pollen tube penetrates through the cuticle layer of the papillar cell wall and penetrates stigma at the base of the papillae. Growing pollen tube also shows remarkable polarity which is generally evident in the form of four distinct regions (Fig. 26.7a). The apical region or tip of the growing pollen tube is free from any major organelles and is referred as "clear zone" (Fig. 26.7b). Within the clear zone, no cytoplasmic streaming can be observed, whereas in the subapical region, it does exist. The subapical region is highly vesicular but is devoid of cell organelles. Actin cytoskeleton supports the intracellular trafficking of secretory vesicles and other organelles along the axially oriented actin throughout the length of the elongating pollen tube. These organelles and secretory vesicles move toward the tip along the edge of the pollen tube, and after reaching the subapical region, they migrate backward toward the pollen through the center of the pollen tube. This type

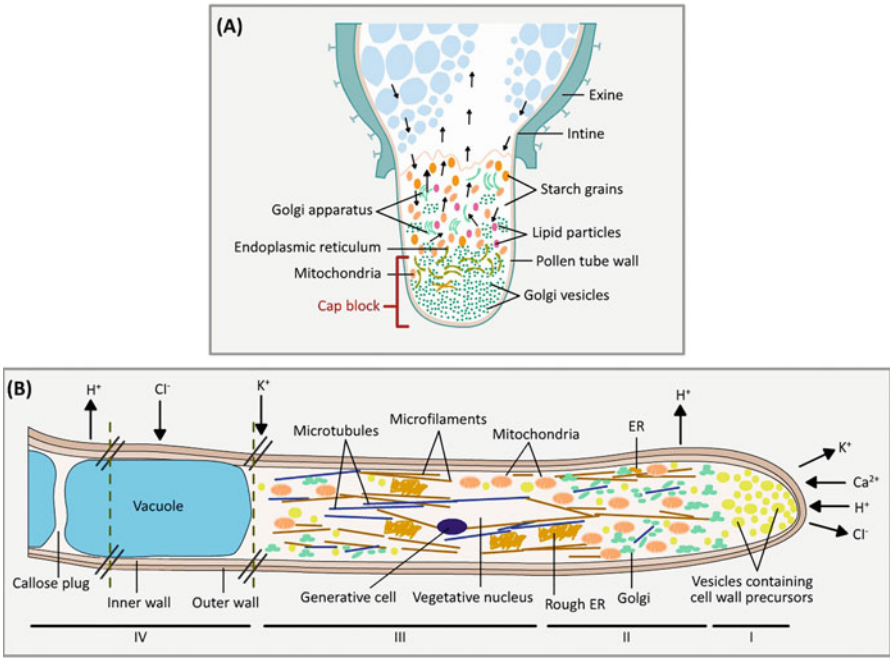


Fig. 26.7 (**a**) Diagrammatic sketch of the tip of pollen tube showing "cap block" region in the just emerging germ tube and a pollen tube. (**b**) Four different zones (I–IV) in the pollen tube. I apical zone, II subapical zone, III nuclear zone, IV vacuolization zone

of movement gives rise to a reverse pattern of cytoplasmic streaming. Actin microfilaments extend to the subapical region but do not invade the clear zone (Fig. 26.8a). Clear zone contains short actin bundles. The base of the clear zone is characterized by the randomly oriented dense mesh of short actin filaments. This rapid actin remodeling at the subapical region is supposed to be important in directing reverse cytoplasmic streaming. Existence of clear zone seems to be related to the disruption and reorganization of actin microfilaments implicated in cytoplasmic streaming.

Actin dynamics is regulated by several actin-binding proteins. These include the G-actin-binding protein **profilin**; the G- and F-actin-binding proteins or the **actin-depolymerizing factors (ADFs)**, also known as **cofilins**; and others that affect different aspects of actin polymerization and higher order organization. In pollen tubes, increasing the level of profilin or ADF results in the disruption of the normal actin cytoskeleton organization and inhibition of pollen tube growth. The small secretory vesicles are involved in delivery of wall materials and membrane to the growing tip. Nuclear region containing two sperm nuclei and large cell organelles (such as mitochondria and endoplasmic reticulum) is present behind the subapical region. Lastly, vacuolar region contains a large vacuole to restrict the backward flow of cytoplasm and sperm nucleus inside the pollen. It is hypothesized that Ca^{2+} and

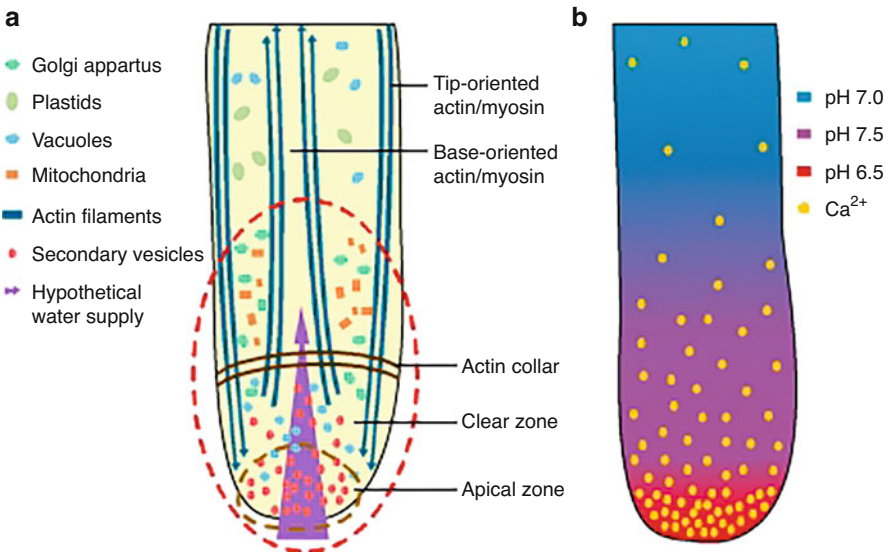


Fig. 26.8 (**a**) Model to show the reverse pattern of cytoplasmic streaming. (**b**) Role of Ca^{2+} and pH gradient in regulation of polarity inside growing pollen tube

pH gradients in the growing tip are involved in the regulation of polarity inside growing pollen tube (Fig. 26.8b). At the extreme tip of the pollen tube, the concentration of cytosolic Ca^{2+} is high (3–10 μM) and drops drastically (0.2–0.3 μM) beyond 20 μm from the tip region. Additionally, the extreme tip region is characterized by slightly acidic cytosolic pH (pH 6.8) and an alkaline pH (7.5) at the interface of the apical and subapical regions. Periodic oscillation in $[Ca^{2+}]_{cyt}$ concentration and pH correlates with changes in the growth rate of pollen tube, pointing toward a possible link between them.

26.3.4 Signaling Events at the Tip of Growing Pollen Tube

A unique family of small guanine trinucleotide phosphatases (GTPases), known as Rho-like GTPase (ROP), is implicated in the regulation of pollen tube tip growth. Basically, GTPases act as molecular switches involving interconversion of GTP (bound active form) and GDP (bound inactive form). Guanine nucleotide exchange factors (GEFs) are responsible for the activation of the inactive GTPases by replacing GDP with GTP and triggering a downstream signaling cascade. GEFs themselves are activated by receptor-like kinases (RLKs). Inactivation of active GTPases is brought about by GTPase-activating proteins (GAPs). In *Arabidopsis*, seven (ROP1, ROP3, ROP5, ROP 8, ROP9, ROP10, and ROP11) out of total 11 different ROP genes are expressed in pollen grains. Levels of ROP1 transcripts are much higher as compared to ROP3 and ROP5, suggesting that ROP1 plays a

Table 26.6 Some facts about ROP in *Arabidopsis*

1. Total 11 isoforms (ROP1–ROP11) have been reported in *Arabidopsis*
2. Seven isoforms (ROP1, ROP3, ROP5, ROP8, ROP9, ROP10, and ROP11) are expressed in pollen grains
3. Level of ROP1 transcript is higher in the tip of pollen tube
4. ROP1 is localized to the apical region close to the plasma membrane of the growing pollen tube.
5. Other ROPs are mostly cytosolic

dominant role during pollen tube tip growth. Immunolocalization studies on pea pollen tubes have demonstrated that a fraction of ROP1 is localized to the apical region of the plasma membrane of the growing pollen tube. However, majority of the ROPs are cytosolic (Table 26.6). It is believed that ROP1 activates at least two downstream signaling pathways that regulate the generation of the tip-focused $[Ca^{2+}]_{cyt}$ gradients and the assembly of dynamic, tip-localized actin microfilaments. ROP1 activates two direct downstream targets, RIC3 and RIC4, which are CRIB motif-containing ROP-interacting proteins. RIC3 modulates the formation of the tip-focused [Ca^{2+}]cyt gradient probably through the regulation of extracellular Ca^{2+} influxes, whereas RIC4 promotes the assembly of the apical F-actin. RLK, upon activation by an unidentified ligand, activates GEF which ultimately activates ROP1. Activated ROP1 stimulates NADPH oxidase (NOX) activity, resulting in the production of reactive oxygen species (ROS). In turn, ROS promotes influx of Ca^{2+} from the extracellular space which enhances tip growth (Fig. 26.9). Actin dynamics is exhibited as periodic fluctuation of F-actin at the tip of the pollen tubes. Dynamics of the apical F-actin microfilaments is not only required for polarized growth of pollen tube but is probably also important for growth oscillations inside the pollen tube. In tobacco pollen tube, overexpression of RIC4 leads to stabilization of actin microfilaments at the tip region, resulting in depolarized growth along with loss of growth oscillations. Since, the activation of the RIC4-dependent actin pathway depends on interaction of RIC4 with active ROP1 at the plasma membrane of the tip region, it can be speculated that ROP1 activity at the tip controls actin dynamics. Cycling between GTP-bound active and GDP-bound inactive status of ROP1 is critical for normal tip growth of pollen tubes. Therefore, it can be proposed that periodic up- and downregulation of ROP1 activity might be required for the modulation of polarity and growth oscillations inside the growing pollen tube.

26.3.5 Directional Growth of Pollen Tube in the Pistil

Once the growing pollen tube penetrates the stigmatic tissue, the subsequent growth inside the pistil is required to be directional. This directional growth along the pistillar path is necessary for the pollen tube to enter the micropyle to effect fertilization. In fact, not one but several pollen tubes enter through the stigmatic tissue and compete for fertilization. Once fertilization takes place, all other pollen tubes stop growing further. The pollen tube growth guidance leading to fertilization

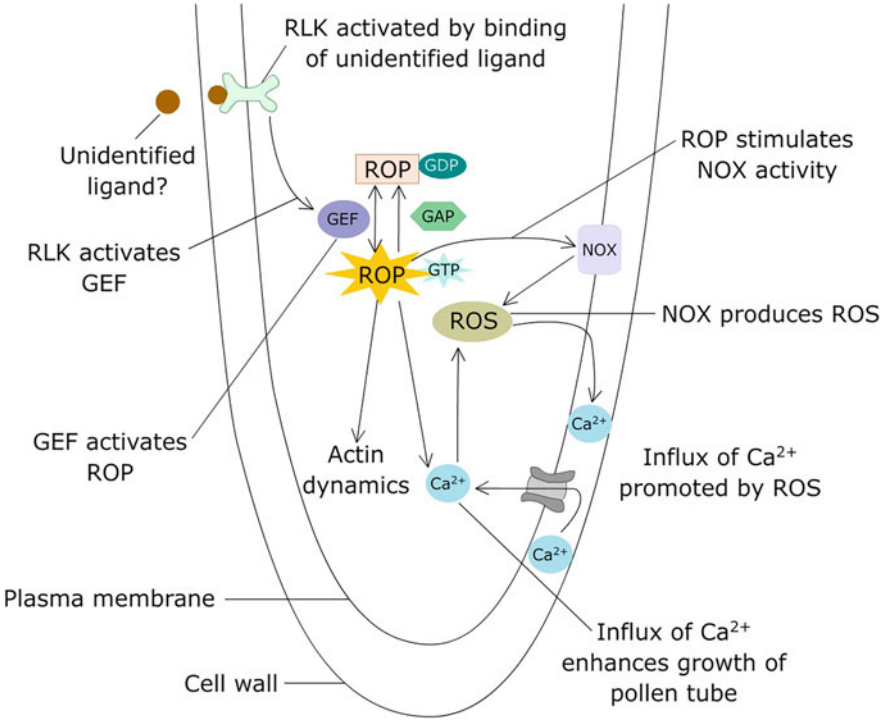


Fig. 26.9 Signaling events at the tip of growing pollen tube

is dependent on the interaction of pollen tube with female tissues. A wide array of biomolecules has been implicated in this interaction process. The directional growth of pollen tube toward the female gametophyte has been explained with the help of two major models: **the chemotropic hypothesis** and the **mechanical hypothesis**. According to chemotropic model, a sequential arrangement of specific biomolecules directs pollen tube toward the ovule. In *Arabidopsis* and lily, plantacyanins (a type of phytocyanins, the blue copper proteins) have been reported to provide the initial **chemotactic guidance**. **Plantacyanins** are secreted by the stigmatic cells which line the transmitting tissue tract in the wet type of stigmas. Experiments involving in vitro pollen tube growth have shown that plantacyanins can reorient the growth of pollen tubes suggesting that they act as chemotactic cues. Overexpression of plantacyanins in the stigmatic papillar tissue makes pollen tube insensitive to the transmitting tissue. As a result, pollen tubes lose their orientation leading to loss of directional growth. Another member of phytocyanin family, **chemocyanin**, also acts as a directional cue. In lily, stigma/style c**ysteine-rich adhesin (SCA)**, a lipid transfer protein, secreted by the transmitting tissue is also reported to guide growth of pollen tube. In *Arabidopsis*, embryo sac produces the chemotactic cues for pollen tube guidance. Mutants which lack female gametophyte show disrupted pollen tube

Table 26.7 Biochemical regulators responsible for directional growth of pollen tube in the pistil

S. No.	Biomolecules	Released from	Detected in
1.	Plantacyanins	Cells lining the transmitting tissue tract	*Arabidopsis*, lily
2.	Chemocyanin	Stigma	Lily
3.	Stigma/style cysteine-rich Adhesin (SCA)	Transmitting tissue	Lily
4.	Cysteine-rich proteins (CRPs), namely, LURE1 and LURE2	Synergid cells	*Torenia* sp.
5.	Defensin-like CRP	Embryo sac	*Zea mays*

guidance. Laser ablation of female gametophyte has shown that synergids serve as a source of the chemotactic signals in attracting competent pollen tubes toward the egg apparatus. In *Torenia*, the two synergid cells on the side of the egg cell emit a diffusible, species-specific signal to attract pollen tube at the last step of pollen tube guidance. It has been reported that secreted, **cysteine-rich proteins (CRPs),** a subgroup of defensin-like proteins derived from the synergid cells, serve as pollen attractants. Two CRPs, namely, LURE1 and LURE2, predominantly expressed in the synergid cells, are secreted on the surface of the egg apparatus. Moreover, they show in vitro activity to attract competent pollen tubes of their own species. In *Zea mays*, downregulation of *ZmEA1* gene expressed in the synergids and the egg cell plays role in micropylar guidance of pollen tube. Table 26.7 summarizes various biochemical regulators responsible for directional growth of pollen tube in the pistil in different model systems. According to the mechanical hypothesis, structure and organization of pistil tissue navigate the path of pollen tube using molecular factors. After entering the stigma surface, pollen tube interacts with **extracellular matrix (ECM)** of the transmitting tissue. ECM is a complex mixture of cell wall proteins, which include arabinogalactan proteins, hydroxyproline-rich proteins and proline-rich glycoproteins. According to mechanical guidance, these proteins serve as adhesive molecules to keep the pollen tube in place, and they also act as navigators for the growth of pollen tube toward the micropyle. ECM also provides nutrients for supporting metabolic activities inside the pollen tube.

Pollen tube reception in the micropyle and subsequent rupture inside the synergid require active communication between pollen tube and embryo sac. After entering the micropyle, pollen tube penetrates one of the synergids (designated as **receptive synergid**) through filiform apparatus and ruptures inside it to release two sperm cells (Fig. 26.10). The receptive synergid undergoes a programmed degeneration process, probably to set up cellular adjustments and reduces its turgor pressure to allow pollen tube discharge. The initiation of degeneration of receptive synergid takes place soon after pollination process, pointing toward a long distance signaling mechanism. In the beginning, the rupture of pollen tube inside the synergid was thought to be driven by the mechanical stimulus resulting from change in osmolarity, leading to pollen tube burst. **FER** gene in *Arabidopsis feronia* mutant encodes a **receptor-like kinase** which is expressed in the synergid cells and accumulates around the filiform apparatus. Probably, the ligand (as yet unknown) for FER is present on the surface

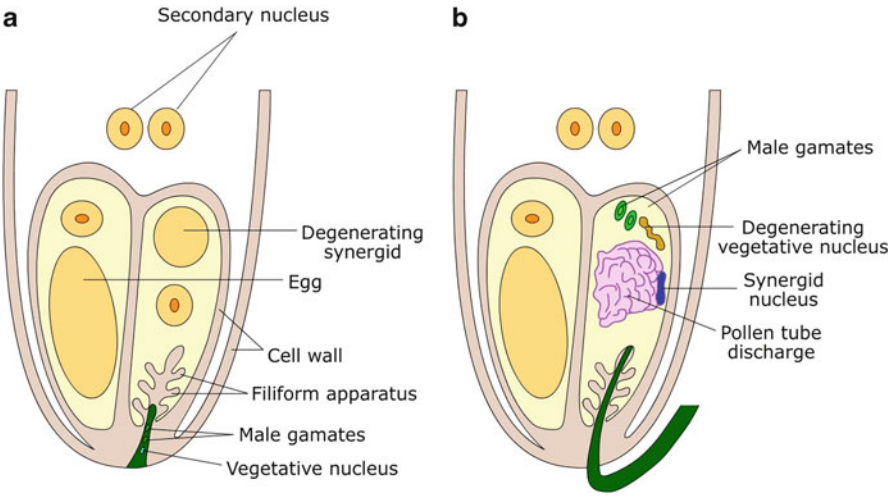


Fig. 26.10 (**a**) Entry of the pollen tube inside the embryo sac through micropyle and its penetration into one of the synergids for delivery of sperm cells. (**b**) Delivery of male gametes and other pollen tube contents into the degenerating synergids

of pollen tube. Furthermore, effective fertilization also depends on the precise timing of pollen tube rupture. In *Arabidopsis*, two homologs of FER, the **ANXUR (ANX1/2) RLKs**, are exclusively expressed in pollen. In anx1/2 mutants, pollen tubes rupture before reaching the egg apparatus, suggesting that ANX1 and ANX2 are male factors controlling pollen tube behavior by directing rupture at proper timing. Furthermore, ANX1 and ANX2 have been reported to be the most closely related paralogs of FERONIA/SIRENE which control pollen tube behavior in the synergid cells. Rupture of pollen tube also depends on various ions, such as Ca^{+} and K^{+} which probably change the osmolarity of pollen tube leading to its lysis. In *Arabidopsis*, mutation in ACA9 gene affects the autoinhibition of Ca^{2+}-ATPase activity due to which pollen tube enters the micropyle but fails to burst and discharge its content, thus suggesting the involvement of Ca^{2+} transport. In maize, ZmES4 (a defensin-like CRP) is exclusively expressed in the embryo sac. Experiments have shown that in vitro application of ZmES4 causes opening of the KZM1, an inward-rectifying K^{+} channel. Downregulation of ZmES4 leads to failure of pollen tube discharge without affecting the guidance.

26.3.6 Double Fertilization

After bursting of the pollen tube inside the receptive synergid cell, the two sperm cells may either remain stationary at the boundary region between the egg and the central cell for some time or fuse with them immediately to initiate seed development. One sperm cell fuses with the egg (syngamy), and the other one fuses with the

central cell (triple fusion) to complete the process of double fertilization. Double fertilization is considered to be unique to angiosperms but formation of two zygotes has been reported in two gymnosperms as well, namely, *Ephedra nevadensis* and *Gnetum gnemon*. It is proposed that male gametes, after their release in the embryo sac, exchange signals with the female gametes for preparation of fusion. In *Arabidopsis*, generative cell specific1 (GCS1) gene expressed in pollen grain has been shown to be required for fusion of gametes. Mutants defective in GCS1 gene exhibit normal movement of sperm cell after discharge of pollen tube content, but do not fuse with female gametes. Moreover, GCS1 gene is highly conserved and has been reported to play a similar role in *Chlamydomonas* (unicellular green alga) and *Plasmodium falciparum* (malaria parasite). Additionally, gametic union is facilitated by the secretion of a CRP by the egg cell (upon arrival of sperm cell) and surface protein by the sperm cell in response to it. Mutants lacking the surface protein in sperm cells are unable to fuse with both egg and central cell. In maize, fusion of sperm cell and egg has been reported to trigger Ca^{2+} influx at the site of fusion, and a wave of Ca^{2+} spreads throughout the egg. Addition of the Ca^{2+} ionophores also causes Ca^{2+} influx in addition to secretion of cell wall components, indicating that Ca^{2+} is functionally important during the phase of fusion of gametes. The two sperms may or may not be equivalent when it comes to functionality. Which sperm cell will fuse with egg and which one with central cell is variable in different model systems. In *Plumbago zeylanica*, which exhibits sperm dimorphism (the two sperm cells differ morphologically in size and number of cell organelles), the smaller sperm cell always fertilizes the egg cell. In *Arabidopsis*, the isomorphic sperm cells are functionally equivalent.

26.4 Pre-zygotic Barriers to Self-Fertilization

Majority of the flowering plants (~85%) are bisexual. Thus, it is presumed that these must be self-fertilizing. However, floral morphology in most of the plants favors attracting pollinators which are instrumental in effecting cross-pollination and not self-pollination. Self-pollination is prevented in both bisexual and monoecious species through spatial and temporal features. Maturation of stamens and pistils at different times, known as **dichogamy**, prevents self-pollination. Dichogamy is of two types-**protandry** and **protogyny.** In protandry, stamens mature before pistils, whereas in protogyny maturation of pistils precedes that of stamens. Another spatial factor, **herkogamy**, i.e., spatial separation of pollen and stigma, reduces chances of self-pollination. An extreme case of spatial separation is exhibited by monoecious and dioecious species in which stamen and pistil are present in the same flower or in different flowers, respectively. In flowering plants, several steps prior to double fertilization are crucial for determining the reproductive success. Not only interspecific hybridization is prevented in many plants through various mechanisms but self-fertilization is also prevented by majority of species. Self-fertilization leads to inbreeding depression due to expression of deleterious traits as a result of high levels of homozygosity in a population. However, self-fertilization is beneficial at times as

it circumvents the necessity of a partner for effecting reproduction. Furthermore, many pioneer plants are self-fertilizing but phylogenetic studies have shown that most of the self-fertilizing species are evolutionarily young. The journey of pollen tube toward the female gametophyte depends on its intricate and highly regulated interaction with the pistillar tissue at various stages, beginning from adhesion and hydration of pollen grains on the stigma surface. This interaction proceeds with germination of pollen grain on stigma surface continuing with penetration of stigmatic surface and growth through the transmitting tissue and style and ultimately entry of pollen tube inside the embryo sac. Thus, pistil acts not only as a conduit for pollen tube growth but also acts as sieve for screening of incompatible pollen grains. This discrimination between compatible (nonself) and incompatible (self) pollen grains is exercised in highly prevalent self-incompatibility (SI) systems which ensure outcrossing by preventing selfing.

26.4.1 Genetic Basis of Self-Incompatibility (SI)

More than half of the angiosperm species (~125,000) have evolved self-incompatibility (SI) mechanisms. SI refers to the inability of the self-pollen to effect successful fertilization in the pistil. It encompasses a variety of diverse molecular and evolutionarily unrelated mechanisms that prevent self-fertilization. In 1925, E M East and A J Mangelsdorf, based on their studies on *Nicotiana*, reported that SI is determined by a single self-recognition locus, known as s**terility or S-locus**. The S-locus has several alleles (S_1, S_2, S_3,. . .. .S_n), and incompatibility results whenever pollen and pistil carry the same allele. S-locus is highly polymorphic such that every allele differs in sequence from the other. The genes implicated in SI recognition must be inherited together in a tight genetic linkage and by suppressing recombination between two components of S-locus. On the basis of genetic behavior, SI can be of two types: **gametophytic self-incompatibility (GSI)** and **sporophytic self-incompatibility (SSI)**. In GSI systems, SI is determined by the genotype of the haploid pollen grain itself, i.e., if the allele carried by the pollen matches with any one of the two S-alleles carried by the pistil, then it is rejected (Fig. 26.11a). In these systems, though pollen grain germinates and penetrates the stigmatic tissue, it gets rejected in the upper zone of the style. GSI systems have been reported in more than 60 families, including Solanaceae, Rosaceae, and Papaveraceae. In SSI systems, the incompatibility reaction is determined by the genotype of the diploid parent plant which produces pollen. In this case, either of the two alleles of the S-locus, which are present in the pollen parent, participates in the recognition reaction (Fig. 26.11b). Rejection reaction takes place if any of the two alleles present in the pollen parent match with the female plant. Majority of the SI systems are controlled by genes within a single S-locus. However, in *Secale cereale* and *Ranunculus acris* SI is dependent on two (loci S and Z) and four loci, respectively. Three main mechanisms have been discussed for the explanation of SI encoded by single S-locus. These mechanisms differ with respect to the manner in which self-pollen is rejected, i.e., whether this rejection involves programmed cell

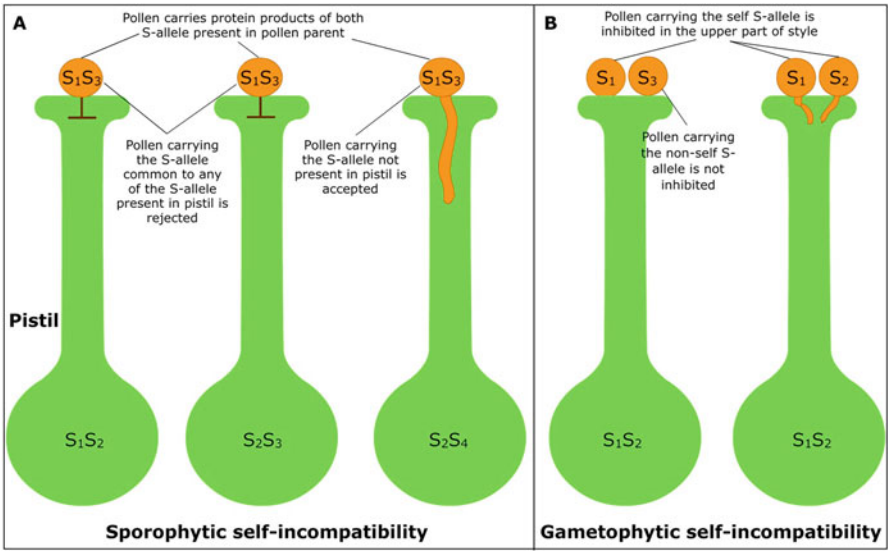


Fig. 26.11 (**a**) Sporophytic self-incompatibility. (**b**) Gametophytic self-incompatibility

death (PCD). The phase and site of pollen rejection, i.e., whether it takes place during early or in the later phase of pollen tube growth through pistil, are determined by the attributes of the stigma surface. For instance, in Brassicaceae, self-pollen is rejected on the surface of dry stigma. Contrary to this, in members of families possessing wet type of stigma, such as in Solanaceae, self-pollen germinates and enters the stigmatic tissue, and inhibition of the pollen tube growth is accompanied by PCD of the pollen grains.

26.4.2 Receptor-Ligand Interactions Mediated Rejection of Self-Pollen at the Stigma Surface

In members of the family Brassicaceae, SI is operational at the level of interaction between pollen grain and stigma surface and is sporophytically determined, i.e., SSI system. The SI reaction is highly localized as it involves only the interface of pollen grain and stigmatic papilla and is observed within minutes of contact between pollen and stigma. This recognition reaction exhibits high degree of specificity such that a single papillar cell of stigma is capable of discriminating between a variety of genetically different pollen grains. As a result, germination of self-pollen grains is inhibited at the surface, whereas nonself pollen grains are allowed to proceed further to effect self-fertilization. Furthermore, incompatible pollen grains inhibited at the stigma surface are capable of forming pollen tubes upon transfer to the surface of compatible stigma. This shows that in members of Brassicaceae family, inhibition of self-pollen does not involve cell death of either pollen grain or stigmatic papilla

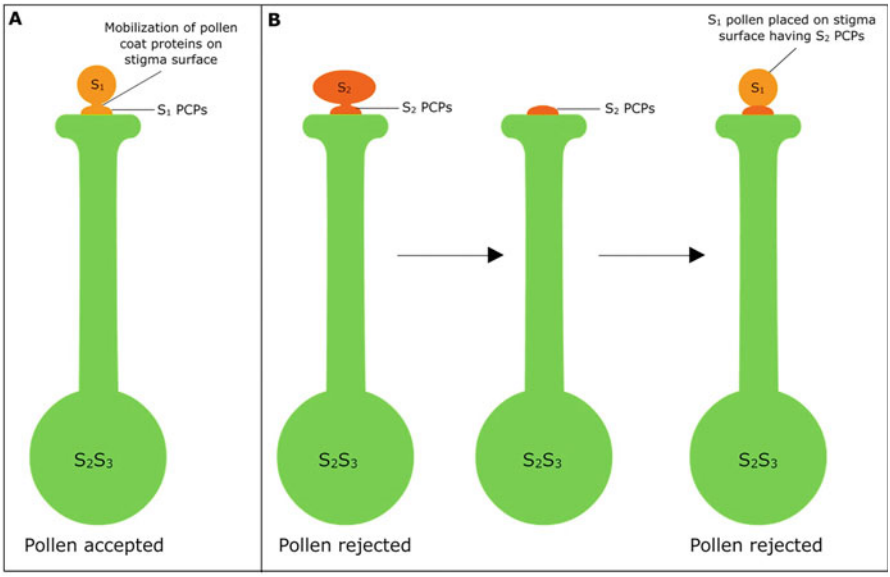


Fig. 26.12 Role of pollen coat proteins (PCPs) in self- incompatibility response

(Fig. 26.12). Once a pollen grain lands on the stigma surface, a part of the pollen coat flows onto the papillar cell. The pollen coat contains the pollen factors that determine the specificity of the SI interaction. This can be demonstrated by placing a compatible pollen grain on to the same spot where an incompatible pollen grain was placed and then removed. The compatible pollen cannot germinate because the pollen coat material that was left behind, initiated an incompatible reaction. The presence of pollen factors in the pollen coat explains the sporophytic nature of SI system in Brassicaceae. The pollen coat contains molecules that were produced by the tapetum. Since the tapetum is diploid, it usually expresses both *S*-alleles of the pollen parent, such that two distinct pollen factors encoded by the two *S*-alleles are expected to be present in the pollen coat.

The mechanism of self-pollen recognition is based on the interaction between cell surface-localized receptors and ligands encoded by two S-locus genes. The S-locus receptor kinase (SRK) gene encoding a single-pass transmembrane serine/threonine kinase, localized in the plasma membrane of the stigmatic papilla, interacts with the S-locus cysteine-rich (SCR) gene (also known as S-locus protein 11, i.e., SP11), encoding a small peptide localized on the pollen coat (Fig. 26.13). SRK and SCR are highly polymorphic so that the amino acid sequence for different alleles can vary up to 35% and 70%, respectively. Initial contact between pollen and stigmatic papillae facilitates mobilization of SCR and other pollen coat substances to the papillae, resulting in the interaction of SRK and SCR, which act as ligand for binding with receptor kinase. The specific interaction between SRK and SCR variants encoded by the common S haplotype is responsible for species specificity of this SI system.

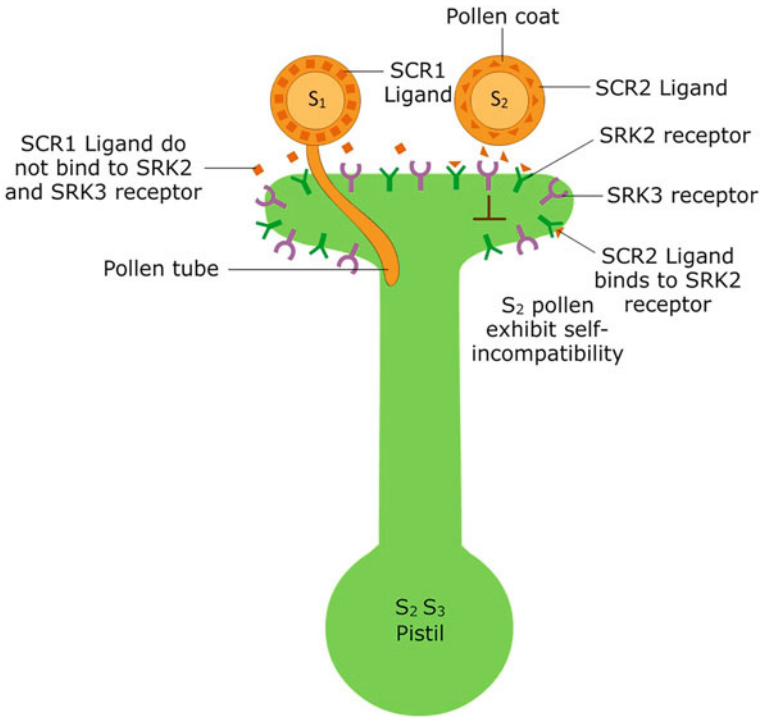


Fig. 26.13 Interaction of receptor and ligand present on the surface of pollen and stigma, respectively, showing rejection of self-pollen (S_2) at stigma surface. SRK, S-locus receptor kinase; SCR, S-locus cysteine rich

26.4.3 Programmed Cell Death of the Pollen Tubes After Penetration into Stigma Surface

Contrary to SSI system in Brassicaceae in which SI response takes place early, *Papaver rhoeas*, which possess GSI system, shows SI response after pollen germination on stigma surface, and leads to the death of pollen tube after its entry into the stigma tissue. GSI system has been characterized in detail in *Papaver*; however, only the female determinant of the SI has been reported. The ligand reported to induce death of pollen tube is a 15 kDa glycoprotein secreted by stigma. This protein is designated as PrS (*Papaver rhoeas* stigma S) which exhibits polymorphism, causing inhibition of pollen tube growth in allele-specific manner. PrS does not show similarity to any protein with known function. However, self-PrS protein causes rapid influx of Ca^{2+} just behind the tube tip which disturbs the cytosolic Ca^{2+} gradient at the tip of pollen tube required for its growth. Initial inhibition of the pollen tube growth is due to actin depolymerization leading to disruption of actin cytoskeleton. This is followed by calcium-/calmodulin-dependent phosphorylation of a 26 kDa inorganic pyrophosphate (p26, IPP) and a 56 kDa mitogen-activated protein kinase (p56, MAPK) found in pollen. Subsequent activation of MAPK

cascade, involving activation of caspases, leakage of cytochrome c from mitochondria into the cytosol and DNA fragmentation, leads to PCD of pollen tube. The putative receptor of PrS protein has been reported to be a novel transmembrane protein of 21 kDa and is designated as PrpS (Papaver rhoeas pollen S). Downregulation of PrpS reduces inhibition of pollen tube growth in an allele specific manner, pointing toward the crucial role it plays in rejection of self-pollen. Another integral membrane protein, S-protein-binding protein (SBP), has been reported to bind to PrpS in a non-allele specific manner and facilitate the inhibition of pollen tube growth.

26.4.4 Inhibition by Cytotoxic Stylar RNAses and Degradation of Pollen Tube RNA

Members of several families such as Solanaceae, Rosaceae, and Scrophulariaceae exhibiting GSI system show a different mechanism for inhibition of self-pollen grain. Germination of self-pollen occurs normally on the stigma surface. However, after entry of pollen tube into stigma, SI response is manifested in the upper region of the style where self-pollen tubes show reduction in the rate of their elongation, cell wall thickening, destruction of cell organelles, and loss of plasma membrane integrity, ultimately leading to bursting of pollen tube. These responses are mediated by non-specific ribonucleases, the **S-locus ribonucleases (S-RNases,** present in the pistil. S-RNase is abundant and highly polymorphic pistil-specific glycoprotein which is encoded by S- locus and secreted into the extracellular matrix present along the length of style through which pollen tube navigates its way to ovule. S-RNases are non-specifically taken up by both self and nonself pollen tubes, and allele-specific inhibition of self-pollen tubes takes place later on. The specificity of this SI response subsequent to RNAse uptake by the pollen tube is dependent on the interaction between S-RNase and S-locus-F-box (SLF) protein expressed in pollen tube. SLF, also known as S-haplotype- specific F-box, is a cytoplasmic protein belonging to F-box protein family and functions as a part of E3 ubiquitin ligase complexes. F-box protein confers specificity to the E3 ubiquitin ligase complexes by specific substrate binding and targets them for proteolysis. SLF binds to subunits of the E3 ligase complex and also interacts with S-RNases. The interaction of SLF and S-RNase is non-allele specific as SLF binds to both self and nonself S-RNases. However, interaction of SLF to nonself S-RNase is stronger as compared to that with self-RNAse. Thus, nonself RNAses are ubiquinated and degraded via proteasome pathway. As a result, growth of the nonself pollen tube is not inhibited. Experiments have shown that deletion of SLF gene does not cause constitutive rejection of the self and nonself pollen tubes. Thus, it has been suggested that an unknown RNAse inhibitor binds to all the RNAses, but binding of S-RNase with its cognate SLF does not permit inhibitor binding, rendering protected RNAse to degrade RNA. This model for self-pollen tube inhibition does not highlight the role of three stylar proteins reported to be required for inhibition of self-pollen. These three proteins are HT-B protein, 120 K glycoprotein, and factor 4936. Therefore, another model

has been suggested for S-RNase-based inhibition in GSI systems. According to this model, HT-B protein along with 120 glycoprotein and factor 4936 enters the pollen tube along with the S-RNase. S-RNases are not degraded rather they are sequestered into endomembrane vesicles along with HT-B protein. These endomembrane vesicles break down subsequently during pollen tube growth and release S-RNases in self-pollen tube but remain intact in case of nonself pollen tubes. Breakdown of the endomembrane leads to release of sequestered S-RNases en masses which is too much for effective binding by inhibitor. Consequently, S-RNases are left active for RNA degradation.

26.5 Seed Development

In flowering plants, the diploid zygote develops into the embryo, whereas the fertilized central cell gives rise to endosperm which nourishes the embryo during its development. The process of seed development is quite complex, and for simplicity it can be discussed under three main processes. (1) **Embryogenesis** results in the formation of embryo from unicellular zygote through cell division, cell specialization, pattern formation and organized growth. (2) This is followed by the **development of endosperm** that takes place in parallel with embryogenesis and results in the development of specialized tissue for storage of nutritional reserves and formation of protective layers around the embryo. (3) The last process is desiccation or maturation drying in which the embryo prepares itself to survive long periods of metabolic inactivity in desiccated form.

26.5.1 Embryogenesis

Stages in the embryogenesis are quite variable among different species. In fact, the final overall appearance of the embryo is also species-specific. The **zygote** undergoes divisions in various planes to grow into a small cluster of cells, out of which some part develops into embryo proper and the other part grows into a short stalk-like structure known as **suspensor**. Suspensor pushes the embryo deep into the endosperm and gets crushed during the later stages of embryo development as it is usually ephemeral in nature. The cells at one end of the suspensor continue to divide mitotically and develop into an embryo. Initially these cells are arranged in the form of a sphere giving rise to the globular stage. Eventually, initiation of two primordia at the end of the embryo farther from suspensor marks the onset of heart-stage. Later, embryo assumes the torpedo stage (an elongate cylinder-like appearance) characterized by a short axis consisting of radical (embryonic root), epicotyls (embryonic stem), and hypocotyls (the root-shoot junction). Ultimately, vascular tissue differentiates within the embryo. The epicotyl may bear a pair of small leaves and radical and it often contains several primordia for lateral roots in its pericycle. After maturation, the embryo becomes quiescent and dehydrates partially. The funiculus may break which leave a small scar called hilum. *The detailed*

description of embryo development and its regulation has been discussed in detail in Chapter 24 (Embryogenesis, vegetative growth and organogenesis).

26.5.2 Endosperm Development

The fusion product of second sperm cell with the central cell, i.e., primary endosperm nucleus, develops into endosperm which is a nutritive tissue required for supporting embryogenesis and seed germination. In flowering plants endosperm may be either transient or persistent in nature. *Transient endosperm* proliferates during early stage of seed formation. Later, however, it gets consumed by the developing embryo. In such cases, as exemplified by tomato, tobacco, and *Arabidopsis*, endosperm does not perform any major role as a storehouse of nutrition. Rather, embryo itself stores the nutrients essential for seed germination. In these cases, endosperm is represented by a single layer called as **aleurone layer** by analogy to cereal grains. In *persistent endosperm* (in case of cereals), endosperm contributes as much as 80% of the total seed mass and supports seed germination until the establishment of seedling. Development of embryo and endosperm occurs in a coordinated manner. Their development is influenced by seed coat which encloses both of them. For normal development of seed, the three components of the seed, namely, embryo, endosperm, and integuments, exchanges signals and coordinate development. However, very little is known about the molecular basis of interactions that takes place between them.

Three different types of endosperm development patterns are known in angiosperms. These are cellular, nuclear and helobial types. **Nuclear endosperm** is the most common type. A seed with nuclear type of endosperm development has an initial coenocytic phase and later cellular phase. The PEN (primary endosperm nucleus) undergoes several mitotic divisions which are not followed by simultaneous wall formation, thus resulting in the formation of **syncytial endosperm (coenocyte)**. Early mitotic divisions are synchronized but at later stage, divisions take place at variable rates resulting in differentiation of three distinct zones, namely, micropylar endosperm, peripheral endosperm, and chalazal endosperm (Fig. 26.14a–c). Expansion of the embryo sac takes place after fertilization, and enlargement of the central vacuole pushes the cytoplasm of the endosperm syncytium toward the periphery. At the globular stage of embryo development, the cytoplasm of the micropylar endosperm surrounds the developing embryo. The syncytial cytoplasm of the peripheral endosperm possesses evenly distributed nuclei.

26.5.3 Cellularization of Endosperm

In **cellular endosperm** development, the division of the primary endosperm nucleus is followed by subsequent wall formation. Thus, cellularization of endosperm is in place right from the initial stage of endosperm development. However, in nuclear type of endosperm, cellularization of endosperm takes place during later stages of

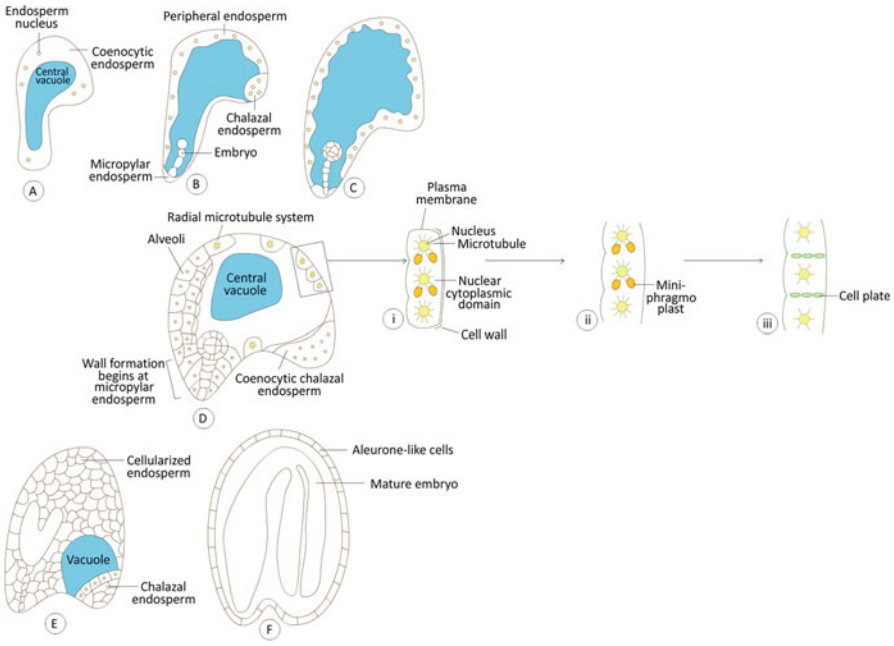


Fig. 26.14 (**a–c**) Repeated divisions of primary endosperm nucleus without cell wall formation and the migration of free nuclei toward periphery of the coenocytic central cell due to enlargement of central vacuole. (**d**) *i-iii* Formation of cross wall in the peripheral endosperm. (**e–f**) Beginning of endosperm cellularization at the micropylar region and later progress toward chalazal region

development. This process of initiation of cell wall formation begins at the globular stage in *Arabidopsis*. Cellularization of endosperm begins in the micropylar embryo-surrounding region (ESR) and progresses toward the chalazal region. Cellularization occurs via formation of radial microtubule systems (RMS) and alveolation (Fig. 26.14d–f). Initiation of cellularization of the coenocytic endosperm begins with the formation of RMS on the surface of all nuclei. Region of the cytoplasm covered by these arrays around each nucleus is known as **nuclear cytoplasmic domain (NCD)**. Subsequently, microtubules from the adjacent nuclei meet to form interzones in which cell wall material, mainly callose, is deposited. Opposing microtubule arrays from the adjacent NCD are known as **cytoplasmic phragmoplasts**, which mediate the deposition of initial cell wall material. Initially, a tubelike structure, known as **alveolus**, is formed by the cell wall material deposited by cytoplasmic phragmoplasts around each nucleus. The open end of the alveolus faces toward the central vacuole. Later, RMS anchors the nuclei to the central cell wall and extends toward the central vacuole in a canopy of microtubules. The interzones between adjacent canopies of microtubules extend the alveoli toward

the central vacuole. Ultimately, after first round of alveoli formation, the nuclei in each alveolus divide periclinally such that the orientation of new cell wall is parallel to central cell wall. These periclinal divisions segregate the alveoli into a peripheral cell and a new alveolus with its opening toward the central vacuole. Repetition of this process 4–5 times results in complete cellularization of the endosperm. At maturity, most of the endosperm is utilized by embryo for its nourishment, and single layer of endosperm is left. This single layer of persistent endosperm is referred to as aleurone layer by analogy to aleurone layer in cereal grains.

Unlike *Arabidopsis*, cellularization of coenocytic endosperm in the case of cereals occurs centripetally. Also, endosperm contributes to a major proportion of mature seed as it is not consumed during embryogenesis. During endosperm development, the triploid PEN undergoes repetitive mitotic divisions which are not accompanied by cell wall formation. This is followed by migration of nuclei toward the periphery of the central cell because of the enlargement of the central vacuole. Similar to *Arabidopsis*, cellularization in cereals proceeds through formation of radially arranged microtubules around each nucleus and alveoli. Formation of anticlinal wall takes place between adjacent nuclei giving rise to tubelike alveolar cells with open end toward the central vacuole. Nucleus of each alveolar cell undergoes 1–2 periclinal divisions accompanied by cytokinesis giving rise to daughter cells. This process takes place in such a manner that the innermost layer of daughter cells remains alveolar in nature. This layer undergoes same round of periclinal division until the cellularization process is complete. After completion of cellularization process, the innermost group of daughter cells undergoes further divisions so that initial cell file pattern is lost. This group of cells is the most important source of starchy endosperm. **Starchy endosperm** constitutes the bulk of endosperm in cereal grains. In addition to starch, starchy endosperm also contains storage proteins stored in protein storage vacuole. For proper development of starchy endosperm, endoreduplication of DNA play a very important role. In maize, endoreduplication leads to up to 96 times increase in DNA content. Often most of the part of cellular endosperm is consumed by the developing embryo. As a result, in a mature seed, embryo occupies the seed completely, and endosperm is represented by one or few layers (i.e., aleurone layer). In maize and wheat, aleurone layer is single layered, whereas barley has three-layered aleurone layer. In rice, the thickness of the aleurone layer varies from one to several layers. In addition to providing nourishment to developing embryo, aleurone layer also acts as major site of mineral storage and confers protection to the nutrient-rich endosperm by expression of stress- and pathogen-protective proteins such as **PR-4 (pathogen-related protein 4)**. During early stages of seedling growth, the aleurone layer mobilizes starch and storage protein from the starchy endosperm by the activity of α-amylase, proteases, and other hydrolases. During seed maturation, the cells of the starchy endosperm undergo programmed cell death. Contrary to this, cells of the aleurone layer survive and acquire desiccation tolerance due to action of abscisic acid. The production of these hydrolytic enzymes takes place in response to gibberellin production by the embryo.

26.5.4 Hormonal and Genetic Regulation of Aleurone Development in Cereal Grains

Early stages of aleurone differentiation are influenced by auxin and cytokinin. In maize, the transgene produced by inducing a mutation in the cytokinin biosynthetic enzyme gene-isopentenyl transferase (IPT) results in mosaic aleurone (interspersed patches of aleurone and starchy endosperm) on the crown regions of kernels. However, the role of endogenously produced cytokinin in controlling aleurone differentiation is still not clear. In contrast, auxin positively influences the aleurone layer. In maize, treatment with auxin transport inhibitor, N-1-naphthylphthalamic acid (NPA) leads to production of kernels with multiple-layered aleurone in contrast to single layer in untreated maize. NPA treatment leads to increased accumulation of auxin in the periphery of endosperm. ABA and GA act antagonistically to mediate the later stages of aleurone development. Aleurone maturation is promoted by ABA, whereas germination is promoted by GA.

Several genes implicated in the control of aleurone layer differentiation have been identified. DEFECTIVE KERNEL1 (DEK1) is essential for aleurone cell fate specification. It encodes for a large complex integral membrane protein localized on the plasma membrane. An extracellular loop present in its structure is suggestive of its potential to interact with extracellular molecules as well as signaling ligands. In maize, a loss-of-function mutation in DEK1 gene results in the production of seeds without aleurone layers. In wild-type maize plants, the cells of the aleurone layer are cuboidal and possess dense granular cytoplasm. In *dek1* mutant, the surface cells present in place of aleurone layer are similar to cells of starchy endosperm and also contain starch grains. Mutation in DEK1 gene results in similar effects in *Arabidopsis* and rice seeds. CRINKLY4 (CR4), a receptor-like kinase, also positively regulates the fate of aleurone layer. *Cr4* mutant, homozygous for recessive allele, shows sporadic patches that lack aleurone predominantly on the kernel. Phenotype of *cr4* mutants resembles those of *dek1-D*, a weak allele of *dek1*. Proteins encoded by these two genes are co-localized in the plasma membrane and in endocytic vesicles. Studies of genetic interactions *cr4* and *dek1* have suggested that these two genes function in overlapping biological processes. Mutation in the SUPERNUMERARY ALEURONE1 (SAL1) gene leads to the formation of multiple layer of aleurone in place of single layer indicating its role in negative regulation of aleurone layer. It exhibits resemblance to human CHMP1, a protein involved in vesicle trafficking. SAL1 protein is also co-localized in endocytic vesicles with DEK1 and CR4. Thus, it has been proposed that SAL1 acts as a negative regulator of DEK1 and CR4 by directing their retrograde cycling off the plasma membrane leading to inhibition of their signaling activity. *Des5* mutant in barley possess single layer of aleurone instead of normal three layers along with variations in characteristic features of aleurone cells, such as larger cell, less dense cytoplasmic contents, and thinner anticlinal walls. *Des5* mutants have drastically reduced cr4 transcript levels. However, reduction in dek1 transcripts was not found to be significant. This

Table 26.8 Mutants defective in aleurone layer differentiation in cereals

S. No.	Mutant	Plant	Phenotype defect
1.	*Dek1*	Maize, *Arabidopsis*, rice	Seeds without aleurone layers
2.	*Cr4*	Maize	Mosaic aleurone due to failure of endosperm to differentiate aleurone
3.	*Sal1*	Maize	Formation of multiple layers of aleurone
4.	*Des5*	Barley	Single layer of aleurone characterized by variations in aleurone cells, such as larger cell, less dense cytoplasmic content, and thinner anticlinal walls

differential expression of CR4 and DEK1 genes in *des5* mutant indicates that these two genes might be independently regulated. Furthermore, expression of the SAL1 gene also decreased in *des5* mutant suggesting that regulation of aleurone cell layer number might be more complex than interplay between cr4 and sal1 functions. Interestingly, relationship between the aleurone layer and epidermis of the leaves has been observed. In maize mutation in CR4 gene not only disturbs the aleurone specification but also disrupts leaf epidermis variously, such as irregular cells with poorly developed cuticle and multiple-layered epidermis. Similarly, weak alleles of dek1 show pronounced effect on the leaf epidermis in maize, rice, and *Arabidopsis*. Table 26.8 summarizes the various mutants defective in aleurone layer differentiation, along with their respective phenotypic characteristics. Quantitative trait locus (QTL) mapping studies in barley have further indicated the possible involvement of some additional factors in determining the number of aleurone layers.

26.5.5 Development of Seed Coat

Following fertilization, differentiation of seed coat or testa takes place from the maternally derived ovule integuments over few weeks (~2–3 weeks in case of *Arabidopsis*). Seed coat forms the outer protective covering surrounding the embryo. At the time of anthesis, mature ovule in *Arabidopsis* consists of two layers of outer integument and three layered inner integument. An endothelium apparently does not form. Instead, the inner integument may be only one cell layer at the micropylar end grading to two and ultimately three cells thick at the chalazal end. Due to cell division and expansion, cells present in all the layers of both the integuments undergo dramatic phase of growth in the initial few days following fertilization. Subsequently, the five cell layers constituting both the integuments follow one of the four distinct pathways. The cells constituting the innermost layer of the integument synthesize proanthocyanidins. These compounds, also known as **condensed tannins**, accumulate in the central vacuole during first week following fertilization. Later, these compounds undergo oxidation and impart brown color to the seed coat.

Contrary to this, the remaining two layers of the inner integument do not differentiate and get compressed ultimately. During the initial phase of growth, cells constituting both layers of outer integument accumulate starch in the amyloplasts. Following this, the cells present in the two layers of integuments follow divergent paths. The subepidermal layer differentiates into palisade cells and produces thickened wall on the inner tangential side of the cells. The epidermal layer synthesizes and secretes abundant mucilage into the apoplast region at the junction of the outer tangential and radial walls. This mucilage is chiefly composed of pectin, and its major constituent is rhamnogalacturonan 1(RG1). This mucilage so deposited acts as specialized secondary cell wall. With the progression of mucilage deposition, contraction of vacuole takes place which leads to the formation of cytoplasmic column in the center of the cell, which is surrounded by donut-shaped apoplastic space filled with mucilage. Subsequently, the space occupied by the cytoplasmic column is replaced by the deposition of secondary cell wall material leading to the formation of columella. During later stages of seed development, the cells of all the layers of seed coat undergo programmed cell death. The structure of the outermost epidermal layer is preserved by mucilage and columella.

In flowering plants, development of seed occurs accompanying complex interactions between maternal tissues, embryo, and endosperm. Growth and differentiation of seed coat are initiated by fertilization, and its development is coordinated with the development of embryo and endosperm. It seems that one or more events during or following fertilization must be involved in transmission of signals to the seed coat that coordinates its development with embryo and endosperm. As already mentioned, in *fis* mutants the process of embryogenesis is blocked. but still development of endosperm and seed coat takes place almost normally. Thus, it appears as if a signal from coenocytic endosperm is sufficient for initiation of seed coat development in the integumentary cells. Furthermore, no significant growth of seed coat takes place in seeds where only egg cell is fertilized. Also, seeds in which endosperm is experimentally destroyed showed inhibition of seed coat development. Recent studies using various mutants have shown that endosperm influences both the growth and differentiation of the seed coat. The growth of seed coat is initiated soon after fertilization and involves both cell division and cell elongation. It has been shown that cell elongation, and not cell divisions, is significant for determining the size of seed. For instance, in *Arabidopsis*, mutation in the HAIKU gene is responsible for limited growth of coenocytic endosperm. This defect also affects the growth of the developing seed coat in such a manner that cell elongation in expanding seed coat is restricted. Thus, it has been suggested that the growing endosperm regulates the extent of cell elongation of the integument following initiation of seed coat development. Contrary to this, loss-of-function mutations in TRANSPARENT TESTA GLABRA (TTG2) gene in *Arabidopsis* restrict cell elongation of seed coat which in turn limits endosperm growth. This "crosstalk" between the developing endosperm and seed coat appears to coordinate growth between the endosperm and seed coat, ultimately establishing seed size.

26.5.6 Seed Maturation and Desiccation Tolerance

Maturation of seed is the final phase of seed development. Hallmarks of seed maturation include accumulation of storage compounds, acquisition of desiccation tolerance, growth arrest, and entry into dormancy period. In several species, **desiccation tolerance (DT)** is also acquired during maturation phase. DT involves drying of seed due to loss of water from seed through evaporation, and it allows the seed to remain dry for extended periods. Operationally, DT is defined as an organism's ability to dry to equilibrium with moderately dry air (50–70% and relative humidity at 20–30 °C) and resumption of normal function upon rehydration. It is basically tolerance to removal of almost total cellular water and its replacement by molecules that are capable of forming hydrogen bonds and substitute interactions in the absence of water molecules. DT is also correlated with seed longevity which determines the ability of the seed to retain its viability over long period of time in desiccated state. Those seeds which are able to tolerate desiccation and can be stored in a dry state for long periods (time period variable depending on species) are known as **orthodox seeds**. The orthodox seeds of *Phoenix dactylifera* were successfully germinated in 2005 after storage for 2000 years. Orthodox seeds acquire DT during seed development. It is commonly initiated along with accumulation of reserves and acquisition of dormancy and is usually fully established just before the drying phase at the end of seed maturation. Mostly, cultivated crops, such as rice, wheat, corn, and barley, produce desiccation-tolerant seeds. Contrary to this, seeds of some plants, such as mango, litchi, cocoa, avocado, and rubber tree, are shed with high water content and active metabolism. Such seeds, known as **recalcitrant seeds**, undergo deterioration upon drying and are not able to survive storage. Table 26.9 lists some examples of orthodox and recalcitrant seeds.

26.5.7 Molecular Basis of Desiccation Tolerance

Acquisition of desiccation tolerance in orthodox seeds is associated with several cellular processes. These include accumulation of disaccharides and oligosaccharides, synthesis of storage proteins, **late embryogenesis abundant (LEA) proteins**, synthesis of **small heat shock proteins (smHSPs)**, activation of antioxidative defense mechanisms, changes in the physical structure of the cell, and gradual increase in density. Variability in desiccation tolerance between different

Table 26.9 Some examples of orthodox and recalcitrant seeds

S. No.	Orthodox seeds	Recalcitrant seeds
1.	*Citrus aurantifolia*	*Persea americana*
2.	*Capsicum annum*	*Theobroma cacao*
3.	*Hamelia patens*	*Cocos nucifera*
4.	*Lantana camara*	*Artocarpus heterophyllus*
5.	*Psidium guajava*	*Mangifera indica*
6.	*Anacardium occidentale*	*Hevea brasiliensis*

plant species is due to physical structure of the seed's internal matrix which involves interactions between sugar and protein complexes with salts, organic acids, and amino acids. Seeds do not possess stomata and have thick seed coat due to which uptake of oxygen and its availability for energy production become limited. Limitation of energy during the period of reserve accumulation becomes more problematic during seed desiccation due to increase in viscosity and molecular packing density of the cells. As a result of these changes, the cells of the embryo are transformed into a glassy matrix. Although seed desiccation refers to physical process of drying, the transition from period of reserve accumulation to seed desiccation is related to significant changes in gene expression as well. This indicates that seed desiccation is also quite an active phase in terms of gene expression. In *Arabidopsis*, expression of ~30% of total genome (6963 genes) changes significantly during desiccation period. Out of these, change in expression of ~21% genes (called as early expressed genes) already begins during the phase of reserve accumulation. Expression of ~43% early expressed genes is upregulated during desiccation, and these include HSPs and LEA genes that are associated with desiccation tolerance.

At molecular level, a strong correlation exists between protective mechanisms such as accumulation of LEA, HSPs, nonreducing sugars, and antioxidants that are activated during dehydration and play a central role in desiccation tolerance. In addition to structural and macromolecular protection, LEA proteins are also involved in the formation of intracellular glassy state and stabilization. Along with nonreducing soluble sugars, LEA proteins have been thought to play an important role in the control of viscosity and mobility properties of these biological glasses in dried state. **Biological glasses** can be defined as highly viscous liquid constituent of desiccated seeds with slow diffusion rate, thus limiting their participation in chemical reactions. Production of ROS is considered to be one of the main reasons for the damage caused due to dehydration. Thus, detoxification of ROS is a critical adaptive mechanism in desiccation tolerance. Many molecular antioxidants, such as ascorbate, glutathione, polyols, tocopherols, quinones, flavonoids, and phenolics, are believed to operate during drying and rehydration to alleviate oxidative stress imposed by desiccation. Several physiological and genetic studies have shown that synthesis of LEA proteins, storage proteins and lipids is promoted by ABA. ABA-deficient mutants fail to accumulate these proteins. ABA treatment induces synthesis of LEA proteins in vegetative tissue. ABA induces changes in cellular metabolism by activating a network of transcription factors either partially or indirectly. The expression of ABA-insensitive (ABI) gene, specifically ABI3, induces the synthesis of LEA and storage proteins through interaction with bZIP (basic leucine zipper) transcription factor such as ABI5. ABI5 gene has been reported to play key role by occupying the central position in the genetic regulatory network and is well connected with LEA and other genes implicated in DT. Thus, it has been proposed that ABI3 and ABI5 along with other genes are the core components of seed-specific ABA signaling pathway which regulate survival during desiccated state.

Summary

- Development of pollen grains takes place in anther locule as a result of two successive mitotic divisions of microsporocyte. During pollen mitosis I (microsporogenesis), formation of vegetative cell and generative cell takes place which is followed by mitotic division of generative cell to form two sperm cells (microgametogenesis). Structure of pollen wall is quite complex and consists of mainly exine (outer) and intine (inner) layer having a pollen coat or tryphine.
- Formation of egg takes place in the female gametophyte through megasporogenesis followed by megagametogenesis. Majority of angiosperms show "*Polygonum* type" of megagametophyte development in which diploid megaspore mother cell undergoes meiosis to produce four haploid megaspores out of which only one undergoes megagametogenesis.
- Transfer of pollen grains to the stigma surface takes place through various biotic and abiotic agencies. Post pollination, pollen grain germinates to form pollen tube which travels through the female gametophytic tissue to deliver sperm cells inside the embryo sac. Formation of pollen tube takes place only after recognition reaction between pollen and stigma. Pollen tubes grow by tip growth triggered by influx of Ca^{2+}. Activation of pollen-expressed receptor-linked kinases by a stigma-expressed unidentified ligand regulates a GTPase switch which enables polar cell expansion of the pollen tube. Directional growth of the pollen tube inside female gametophytic tissue is determined by physical and chemical cues from the pistil and embryo sac.
- After entry of pollen tube inside the embryo sac through micropylar end, the two sperm cells are delivered inside the embryo sac which fertilizes the egg cell and central cell. Double fertilization leads to the formation of diploid zygote (fusion product of sperm cell and egg cell) and primary endosperm nucleus (fusion product of sperm cell and central cell).
- Self-incompatibility (SI) prevents self-fertilization in flowering plants and is determined by S-locus having several alleles. Rejection of self-pollen at the stigma surface is mediated by interaction between S-locus receptor kinase (expressed in stigmatic papillae) and S-locus cysteine-rich protein (expressed in pollen coat). Gametophytic self-incompatibility (GSI), characterized by rejection of pollen tube in the upper part of style, is determined by the genotype of the pollen itself.
- Development of seed includes embryogenesis (development of embryo), development of endosperm, and desiccation tolerance. During embryogenesis diploid zygote undergoes divisions in various planes that eventually gives rise to a mature embryo with well differentiated parts, namely, cotyledon(s), plumule, and radical. Postfertilization, primary endosperm nucleus develops into endosperm which becomes multinucleate (coenocyte) and provides nourishment to the developing embryo. Cellularization of the coenocytic endosperm begins from the micropylar to the chalazal region whereas in cereals cellularization takes place centripetally.

Endosperm development is repressed until after fertilization by FIS proteins which are responsible for methylation and demethylation of DNA and histones in the endosperm. Differentiation of aleurone layer takes place from starchy endosperm cells, and few genes, such as DEK1, CR4, SAL1, and DES5, have been implicated. However, the exact mechanism is yet not understood. Seed coat develops from integuments, and its development is regulated by endosperm.

- Acquisition of desiccation tolerance is facilitated by expression of LEA proteins which are implicated in the formation of glassy state that confers stability to dessicated seed. Synthesis of LEA proteins is regulated by abscisic acid.

Multiple-Choice Questions

1. The term "xenogamy" refers to:
   (a) Transfer of pollen on the stigma surface of the same flower
   (b) Transfer of pollen on the stigma surface of another flower present on the same plant
   (c) Transfer of pollen on the stigma surface of flower present on the different plant
   (d) Fusion of male gamete with the egg in the pistil present on the different plant
2. Which of the following type of stigma surface has not been characterized in flowering plants?
   (a) Dry type
   (b) Wet type
   (c) Semidry type
   (d) Semi-wet type
3. The most common type of embryo sac development observed in angiosperm species is:
   (a) *Oenothera* type
   (b) *Plumbago* type
   (c) *Plumbagella* type
   (d) *Polygonum* type
4. Which of the following statements is incorrect in context of pollen hydration and subsequent germination?
   (a) Influx of Ca^{2+} into vegetative cells.
   (b) Concentration of free cytosolic Ca^{2+} [$Ca^{2+}{}_{cyt}$] increases below germ pore region after pollen hydration.
   (c) Accumulation of actin microfilaments and secretory vesicles takes place below germ pore area prior to pollen tube emergence.
   (d) Following pollen germination, the elongation of pollen tube occurs by lateral growth.

5. The directional growth of pollen tube in pistil is not regulated by:
   (a) Plantacyanins secreted by the cells which line the transmitting tissue
   (b) Stigma/style cysteine-rich adhesin (SCA) secreted by the transmitting tissue
   (c) Cysteine-rich proteins (CRPs) derived from the transmitting tissue
   (d) Extracellular matrix (ECM) of the transmitting tissue
6. Which of the following is not a pre-zygotic barrier to self-fertilization?
   (a) Syngamy
   (b) Dichogamy
   (c) Herkogamy
   (d) Heterostyly
7. Which of the following mechanism is incorrect in context of SI mechanisms?
   (a) In sporophytic SI (SSI) systems, the rejection of self-pollen takes place on stigma surface due to interaction between S-receptor kinase (SRK) and S-locus cysteine rich (SCR) protein.
   (b) In some species exhibiting SSI, the programmed cell death of the pollen tubes takes place after penetration into stigma surface.
   (c) In some species exhibiting gametophytic SI (GSI), the programmed cell death of the pollen tubes takes place after penetration into stigma surface.
   (d) In some species exhibiting GSI, bursting of pollen tubes at the upper region of style due to responses mediated by stylar S-locus ribonucleases (S-RNases).
8. The most common type of endosperm development observed in angiosperms is:
   (a) Nuclear endosperm type
   (b) Cellular endosperm type
   (c) Helobial endosperm type
   (d) Ruminate endosperm type
9. Mutation in Dek1 gene results in:
   (a) Mosaic aleurone due to failure of endosperm to differentiate aleurone
   (b) Formation of multiple layers of aleurone
   (c) Seeds without aleurone layers
   (d) Single layer of aleurone
10. Which of the following species have recalcitrant seeds?
   (a) *Hamelia patens*
   (e) *Mangifera indica*
   (f) *Lantana camara*
   (g) *Capsicum annuum*

Answers

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

Suggested Further Readings

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