FERTILIZATION AND DOUBLE FERTILIZATION

The process of fusion of male gamete with female gamete is called **fertilization** (fecundation). It was first reported by **Strasburger** (1884) in *Monotropa*. In angiosperms, the male gametes are brought to the egg containing female gametophyte by a pollen tube. This phenomenon is called **siphonogamy**. Various events leading to fertilization are described as under :

1. **Germination and Growth of Pollen Grains** (Fig. 7.84). Pollen grains are shed usually at 2-celled stage. The female gametophyte (embryo sac) is deep seated in the ovule and is quite away from stigma. The 2-celled pollen grains reach to the receptive stigma through the agency of wind/animals/water. They remain viable from few minutes to few days in different species. The pollen grains get adhered to the stigmatic surface. For this stigma secretes some **stigmatic fluid** or a sticky oily exudate or mucilage. The pollen grains on stigma absorb water and sugary substances, get hydrated and swell up within few seconds to few minutes and release **wall held proteins** so that recognition factors from pollen and stigma come close to each other and recognise each other. This is **chemotaxy**. The exine ruptures at one of the germ pores and the intine comes out as **pollen tube**. The generative cell divides to form two male gametes. The tube nucleus reaches to tip of pollen tube. Usually one pollen tube arises from one pollen grain (**monosiphonous**) but 14 pollen tubes in *Malva* and 10 in *Althaea* are reported (**polysiphonous**). However, only one of these progresses further and others degenerate. The pollen tube is unbranched but in Amentiferae, branched pollen tube is formed. The pollen tube secretes pectinases and hydrolytic enzymes to create a passage for its entry into solid style. The style provides nourishment to the pollen grain.

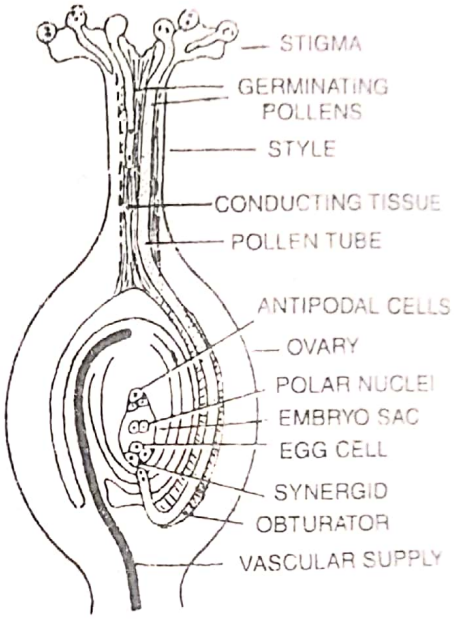


Fig. 7.84. Longitudinal section of a pistil showing growth of pollen tube towards embryo sac through micropyle.

The movement of pollen tube towards embryo sac through style is **chemotropism**. It is due to a **concentration gradient of calcium-boron-inositol-sugar complex**. **Boron** in pollen enhances pollen germination and pollen tube growth. **Calcium** in pistil directs pollen tube growth and pollen germination rate (Taylor *et al* 1997). The aromatic compounds like flavonols derived from tapetum also regulate pollen germination and pollen tube growth. Initial growth of pollen tube takes place on expenditure of food present in the pollen grain and then from stigma and style. Growth of the pollen tube occurs only towards its tip. Depending upon the length of style and passage inside the ovary, pollen tube may reach a length of a few mm to maximum 45 cm (in maize bearing longest style).

Style may be hollow (**open style**, *e.g.*, monocots) or solid (**closed style**, *e.g.*, cotton). Hollow style has a canal lined by special glandular cells. Solid style has a special tissue of pectinised thick walls called **conducting** (transmitting) **tissue**. The pollen tube travels along the lining of canal in hollow style taking nourishment from its cells. In solid style, pollen tube grows through

intercellular spaces between cells of transmitting tissue by separating their cells through secretion of pectinases. The food is absorbed by diffusion. This stylar nutrition together with the fats and proteins in the pollen grain enable a long pollen tube to grow from the pollen grain. The pollen tube forms a path through style to the ovary by **chemotropism**. The pollen tube was discovered by Italian mathematician **Amici** (1824) in *Portulaca*. It behaves as (i) carrier of male gametes, (ii) secretes auxins to stimulate ovary, (iii) haustorial organ. The growth and direction of the pollen tube is **controlled** by the generative nucleus. Growth of pollen tube is limited to the **apical region**, where most of the cytoplasm is concentrated. The remaining part of pollen tube is vacuolated. Many **callose plugs** (Fig. 7.85) are found in the pollen tube at different distances which are responsible to keep the cytoplasm of the pollen tube in apical region. As the pollen tube grows, new plugs are formed continuously at short intervals. These plugs divide the pollen tube into segments and seal the tube. The extreme tip of pollen tube bears a crescent shaped pectin rich transparent area known as **cap block** which regulates growth of pollen tube. The tube grows so long as the cap block exists. In the ovary, the growth of the pollen tube is directed by another tissue called **obturator**, formed by placenta at micropylar end. It grows towards the micropyle and forms a bridge for the pollen tube to approach the ovule. After fertilization obturator shrinks and disappears. The pollen tube moves at the rate of 1 – 6 mm/hour.

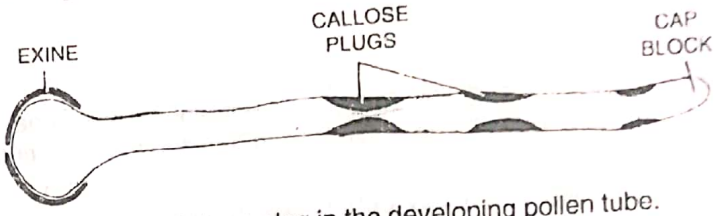


Fig. 7.85. Callose plug in the developing pollen tube.

2. **Entry of Pollen Tube into Ovule**. On reaching the ovary, the pollen tube grows towards one of the ovules. It is attracted by synergids through **filiform apparatus** that secretes some chemicals. The pollen tube enters the ovule through one of the following routes (Fig. 7.86) :

(a) **Porogamy**. It is entry of pollen tube into the ovule through **micropyle**. It is **most common** mode of entry of pollen tube into the ovule, found in orthotropous and anatropous ovules, *e.g.*, Lilies.

(b) **Aporogamy**. It is entry of pollen tube into ovule through any route except micropyle and may be :

(i) **Chalazogamy** (or basigamy). Pollen tube enters the **chalaza** through funicle. It was reported by Treub 1891 in *Casuarina*. Other examples are *Betula, Juglans*.

(ii) **Mesogamy**. When the pollen tube enters the ovule through the sides of the **integuments**, near to placenta, *e.g.*, *Cucurbita, Populus* or through funicle (*e.g.*, *Pistacia*), it is called mesogamy.

(iii) **Acrogamy**. Here pollen tube reaches upto micropylar end through funicle, but do not enter into embryo sac. The embryo sac comes out of ovule to receive pollen tube, *e.g., Utricularia*.

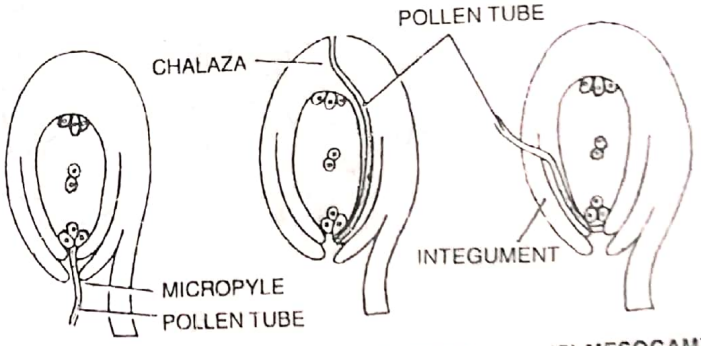


Fig. 7.86. Three modes of entry of pollen tube into the ovule.

3. **Entry of Pollen Tube in the Embryo Sac**. Whatever may be the mode of entry of pollen tube into the ovule (micropyle or chalaza or integument), the pollen tube always enters the embryo sac through the micropylar region where egg apparatus is present (egg + synergids). It is attracted by synergids through filiform apparatus that secretes some attractants (**chemotropic secretion**) like Ca^{++}. The pollen tube tip enters via one of the following four modes (Fig. 7.87) :

(a) Between the egg cell and one of the synergids without destroying the synergids, *e.g.*, *Fagopyrum*.

(b) Between the wall of the embryo sac and egg cell (when synergids are absent) or one or both the synergids, *e.g.*, *Cardiospermum*.

(c) Between the two synergids without destroying either of them.

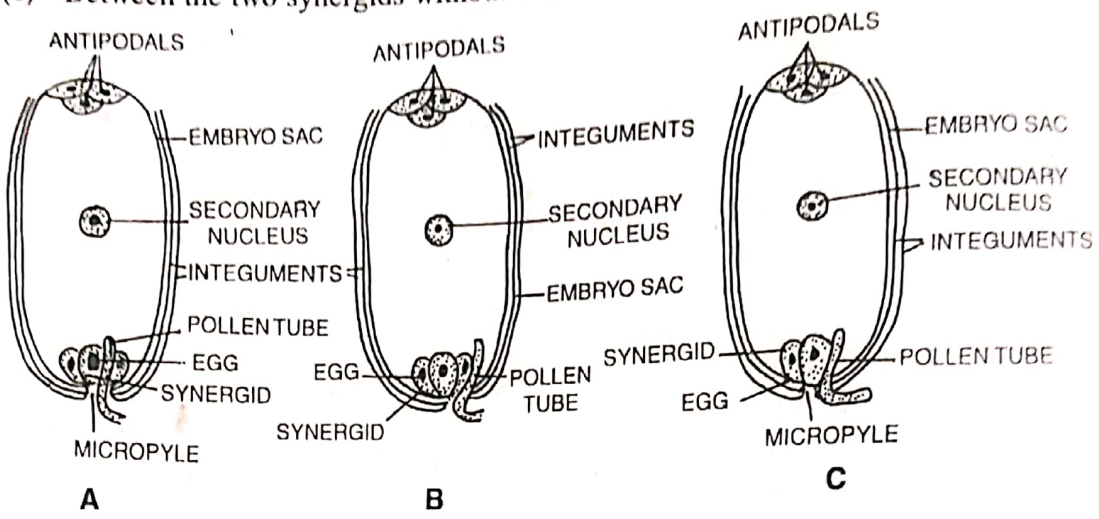


Fig. 7.87. (A, B, C). (A) Pollen tube entering between one synergid and egg. (B) Pollen tube entering between embryo wall and a synergid. (C) Pollen tube entering the embryo sac directly through a synergid.

(d) Directly penetrates one of the synergids through filiform apparatus and bursts open to release its two male gametes into the **synergid** (Fig. 7.88), *e.g.*, *Oxalis*.

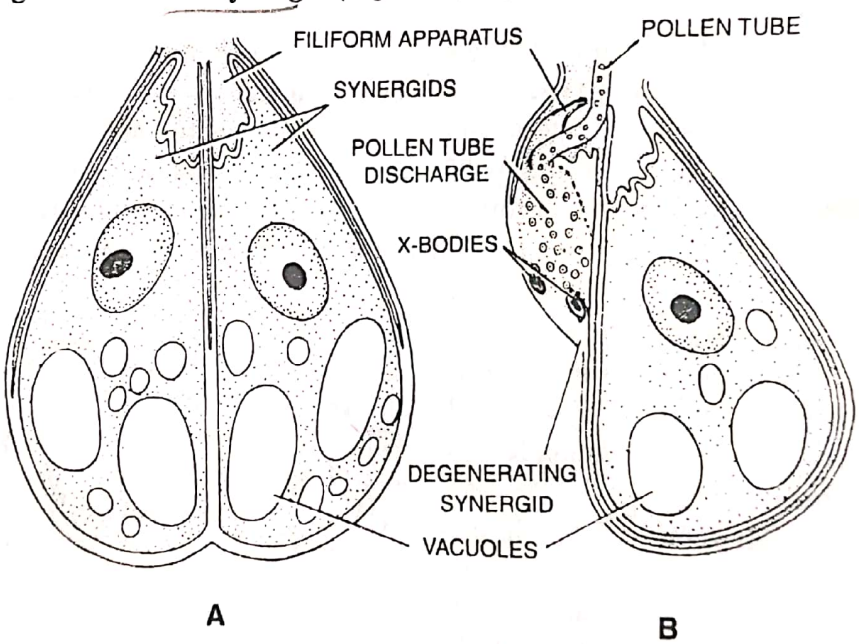


Fig. 7.88. (A, B) Synergids and entry of pollen tube into the embryo sac.
(A) Synergids before the entry of pollen tube. (B) Degenerating synergids after the entry of pollen tube.
[Note that two X bodies represent degenerated tube nucleus of pollen tube and remains of degenerating synergid nucleus.]

As a rule, the penetrated synergid and tube nucleus of pollen tube degenerate (Jensen, 1973). The second synergid also gets destroyed at later stage. The synergid that receives pollen tube degenerates even before the entry of pollen tube into the embryo sac. It starts soon after **pollination** in a number of plants.

4. **Discharge of Male Gametes from Pollen Tube.** Male gametes are discharged from pollen tube into the embryo sac by one of the following methods (Fig. 7.89 A & 89 B) :

(a) Two subterminal openings are produced in the pollen tube, one gamete is discharged through each opening.

(b) The tip of the pollen tube divides into two short branches, one of which is directed towards the egg and other towards the secondary nucleus and then apical ends of each branch burst to release male gamete.

(c) The apex of the pollen tube bursts at the apex releasing both male gametes.

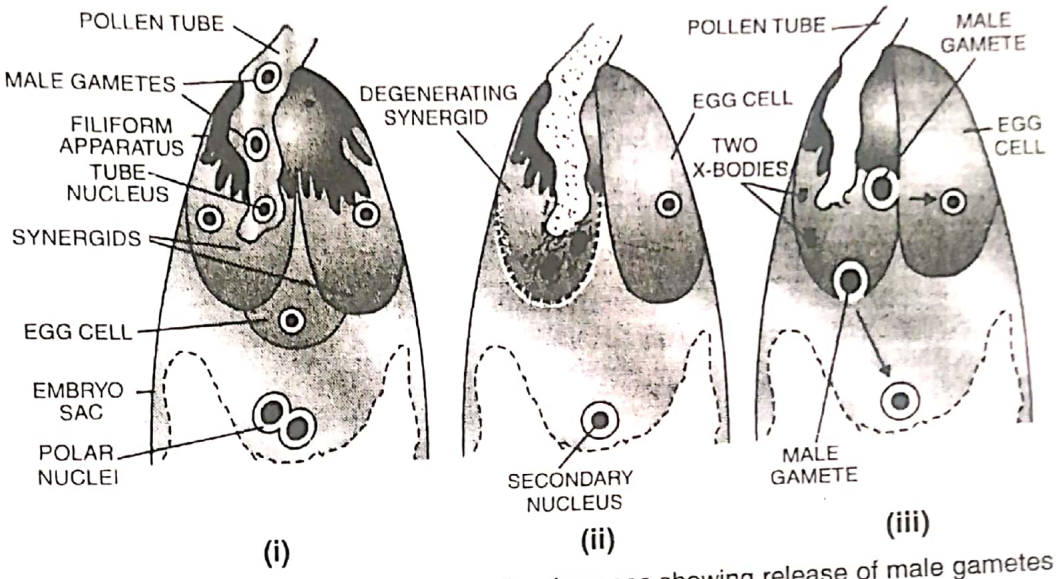


Fig. 7.89. (A– i, ii, iii) Micropylar end of embryo sac showing release of male gametes and the process of fertilization.

The cytoplasm of the synergid and pollen tube do not mix because cytoplasm of synergid lies at micropylar end and that of the pollen tube at chalazal end. Usually only one pollen tube enters in one embryo sac.

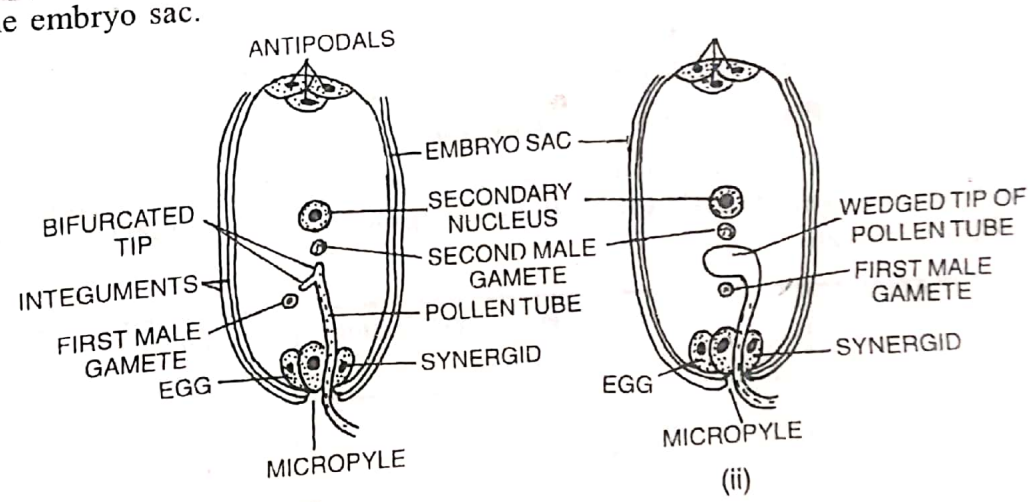


Fig. 7.89. (B–i, ii). (i) Pollen tube tip bifurcated showing discharge of male gametes (*Portulaca*). (ii) Pollen tube tip wedged (*Crepis*) showing discharge of male gametes into central cell of embryo sac.

5. **Movement of Male Gametes (Sperms) toward Egg and Secondary Nucleus in the Embryo Sac.** The contents of pollen tube are released in one of the synergids. Sperms do not have to travel long because the egg cell (oosphere) and synergids lie closely as egg apparatus. The male gametes are carried **passively along the cytoplasmic streaming and amoeboid movement** to the egg nucleus or secondary nucleus in the embryo sac.

6. **Fusion of Gametes** (Fig. 7.90). After discharge from the pollen tube, one of the male gametes (sperm) fuses with egg cell (oosphere) to form diploid oospore (zygote, 2x). This fusion of male gamete with egg cell is called **syngamy** or **true fertilization** or **fecundation** or **generative fertilization** or **amphimixis**. It was reported by Strasburger (1884). The oospore soon after its formation is sur-

rounded by a cell wall and develops into **embryo** after a period of rest. The second male gamete moves down to the centre of the embryo sac and fuses with diploid secondary nucleus (2x, formed by fusion of two haploid polar nuclei) to form triploid (3x) primary endosperm nucleus (PEN). This fusion of male gamete with secondary nucleus is called **vegetative fertilization** or **triple fusion** or **pseudo fertilization** or **second fertilization**. Since three nuclei (one of male gamete and two polar nuclei) are involved, it is called triple fusion. It was reported by **Nawaschin** (1898). This triple fusion converts central cell of embryo sac into primary endosperm cell which later on develops into triploid **endosperm**. Sometimes egg is fertilized with male gamete of one pollen tube and secondary nucleus with male gamete of other pollen tube. This is called **heterofertilization**. In *Allium*, secondary nucleus is haploid and so endosperm will be diploid. In *Fritillaria, Lilium, Plumbago, Peperomia*, the secondary nucleus is polyploid, therefore, endosperm will also be polyploid.

Usually in triple fusion, first, two haploid polar nuclei fuse to form diploid secondary nucleus and then it fuses with male gamete. In some cases like *Fritillaria*, fusion of two polar nuclei is delayed until the male nucleus has joined them, all three then fuse simultaneously. It is interesting to note that behaviour of both male gametes is same and any of them can fuse either with egg or secondary nucleus. Thus both the male gametes participate in fertilization. Thus **fertilization occurs twice in the same embryo sac. It is called double fertilization.**

Pollen tube, antipodals and synergids degenerate after fertilization.

In syngamy or triple fusion, fusion of two nuclei involves following steps :

(i) Close contact of two nuclei;

(ii) Their bordering endoplasmic reticulum fuses at several points and joins their outer nuclear membranes;

(iii) Shrinkage of membranes to form bridges between the nuclei;

(iv) The inner nuclear membranes of the fusing nuclei also merge and their nucleoplasms become continuous at several points and

(v) Finally fusion of two nuclei completely.

Interval between Pollination and Fertilization

The time gap betwen pollination and fertilization is from 2 hours to 12 days or even more. **Triple fusion occurs usually, little bit earlier to syngamy as the cytoplasm of central cell is more active than the egg.**

Plant	Interval between pollination and triple fusion	Interval between pollination and syngamy
Wheat	1.5 – 5 hr	2 – 5 hr
Cotton	24 – 32 hr	24 – 32 hr
Lilies	7 – 8 days	9 – 12 days
Triticale	4 – 5 hr	10 – 15 hr

In many cases, more than two sperms are released in an embryo sac. This is called **polyspermy**. The polyspermy may bring about fertilization of egg by more than one sperm (polyploid zygote) or extra sperm can fertilize synergid or antipodal cells resulting in the formation of more than one embryos in an embryo sac (polyembryony).

Double Fertilization

Each embryo sac receives two male gametes, one male gamete (x) fuses with egg cell (x) to form zygote (2x). This is called syngamy or generative fertilization. Second male gamete (x) fuses with secondary nucleus (2x) to form primary endosperm nucleus (PEN, 3x). This act of fertilization is called triple fusion or vegetative fertilization. *Thus, fertilization takes place twice*

in the same embryo sac. This fusion of two male gametes to two different nuclei of the same female gametophyte (embryo sac) in order to produce two different structures (one is zygote and other is PEN) is called **double fertilization** (Fig. 7.90). It includes syngamy as well as triple fusion. It was discovered by **Nawaschin** (1898) in *Fritillaria* and *Lilium* which later on confirmed by **Guignard** (1899). It is a characteristic of all angiosperms except in members of Trapaceae, Orchidaceae and Podostemonaceae due to lack of hormones. Though double fertilization is also recorded in few gymnosperms like *Ephedra* but here it has no significance and produces no result. Total 5 nuclei (2 male nuclei + one egg cell nucleus + 2 polar nuclei) are involved in double fertilization.

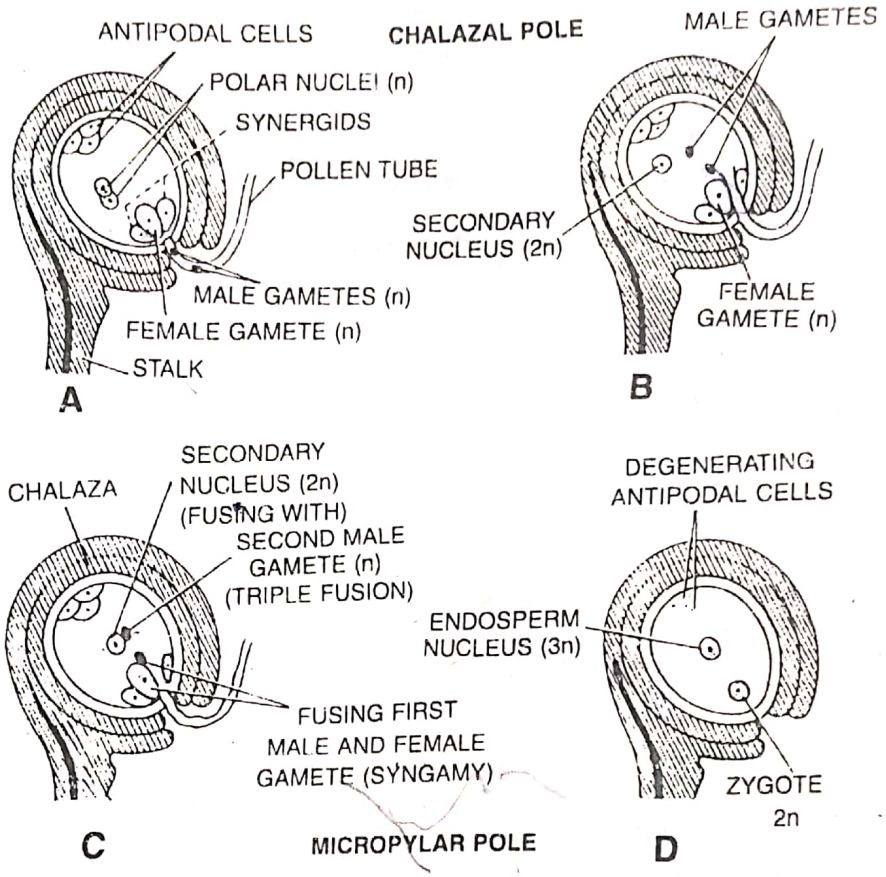


Fig. 7.90. L.S. ovules showing different stages of fertilization and double fertilization.

Significance of Double Fertilization

1. Triple fusion of double fertilization is necessary for the production of viable seeds.
2. It stimulates production of endosperm (a nutritive tissue for developing embryo).
3. Further growth of embryo sac which has stopped at 7 celled stage, is resumed after double fertilization. Triple fusion provides a stimulus to resume growth.
3. It ensures that the endosperm (nutritive tissue) is formed only when the formation of embryo has been ensured by syngamy. This is an advancement over gymnosperm where endosperm is formed long before fertilization. If fertilization fails, the energy spent on forming it shall go waste. In angiosperms, endosperm is formed when needed and this need arises only after zygote formation. If fertilization fails, no endosperm will be formed in angiosperms and no wastage of energy occurs.
4. It provides some characteristic of the male (pollen) plant to the endosperm.
5. It is recorded that seeds without triple fusion are nonviable and embryo formation does not occur.

POST-FERTILIZATION CHANGES AND SEED FORMATION

Soon after pollination, the flower begins to fade. It is sometimes accompanied by sudden increase in respiration and ethylene production. The petals, stamens and style wither away. The calyx may persist (*e.g.*, Tomato, Brinjal) and even show growth as in *Physalis* and *Dillenia*. Other changes which take place are **endosperm formation, embryo development, seed formation** and **fruit formation**.

(A) ENDOSPERM

Endosperm is the food laden triploid (3n) tissue formed as a result of triple fusion of double fertilization and is meant for nourishing the embryo in seed plants. In gymnosperms it is haploid (n), represents the female gametophyte and formed before fertilization. In angiosperms the endosperm is actually formed as a result of **vegetative fertilization** (triple fusion) or fusion of one haploid (n) male gamete with diploid secondary nucleus (2n) of the central cell. Vegetative fertilization gives rise to a primary endosperm cell having a triploid primary endosperm nucleus. The triploid nature of endosperm was given by Nawaschin.

Endosperm formation is a characteristic of all angiosperms except Orchidaceae, Trapaceae and Podostemonaceae. In these forms, triple fusion is completed but the fusion product usually degenerates due to lack of sufficient hormones in the embryo sac. Endosperm in *Crinum, Viscum, Raphanus* is green. Usually, development of endosperm is initiated before that of the embryo.

The number of nuclei contributed by the male gametophyte in the formation of the endosperm is **constant** and one throughout the angiosperms. It is the number of nuclei contributed by the female gametophyte that varies with the type of embryo sac, *e.g.*, *Oenothera*, it is just one and, therefore, endosperm is diploid whereas in *Paperomia*, eight nuclei of female gametophyte fuse with one nucleus of male to form 9n endosperm.

Depending upon the mode of its formation, angiospermic endosperm is of three types—nuclear, cellular and helobial (Fig. 7.91).

1. **Nuclear Endosperm**. The primary endosperm nucleus (PEN) divides repeatedly without wall formation, *i.e., free nuclear divisions* to produce a large number of free nuclei. A central vacuole appears in the central cell and pushes the cytoplasm containing the nuclei to the periphery. The cytoplasm thickens so that the vacuole decreases in size. It ultimately disappears with the exception of a few cases. The multinucleate cytoplasm undergoes cleavage and gives rise to a multicellular tissue. Actually, after the first few divisions of primary endosperm nucleus, a peripheral layer of cells is formed. These cells divide periclinally until the whole embryo sac cavity is filled with the cells of the endosperm. This is most common method of cellularization of endosperm. In some cases, these peripheral cells elongate and extend **centripetally** until they meet in the middle and then each elongated cell divides into many small cells *e.g.*, *Fritillaria*, Maize, Wheat, Rice, Sunflower, *Capsella bursa-pastoris*. In several cases, the cell wall formation remains incomplete. For example, Coconut has multicellular endosperm in the outer part (coconut meat) and free nuclear endosperm in the centre as coconut milk (**liquid syncytium**). In *Phaseolus* wall formation occurs around the embryo only while in *Crotalaria* it is restricted to the upper half.

Nuclear endosperm is the **most common type of endosperm**. It is found in about 56% of families of angiosperms. It is named so because it contains free nuclei in the beginning.

2. **Cellular Endosperm**. Every division of the primary endosperm nucleus is followed by cytokinesis. Therefore, embryo sac/endosperm becomes cellular from the very beginning. First division is usually transverse. **Haustoria are very common** in this endosperm and hence it is primitive type of endosperm. It is found in 25% families of angiosperms, **mostly dicots**, *e.g.*, *Balsam, Datura, Petunia, Drimys*.

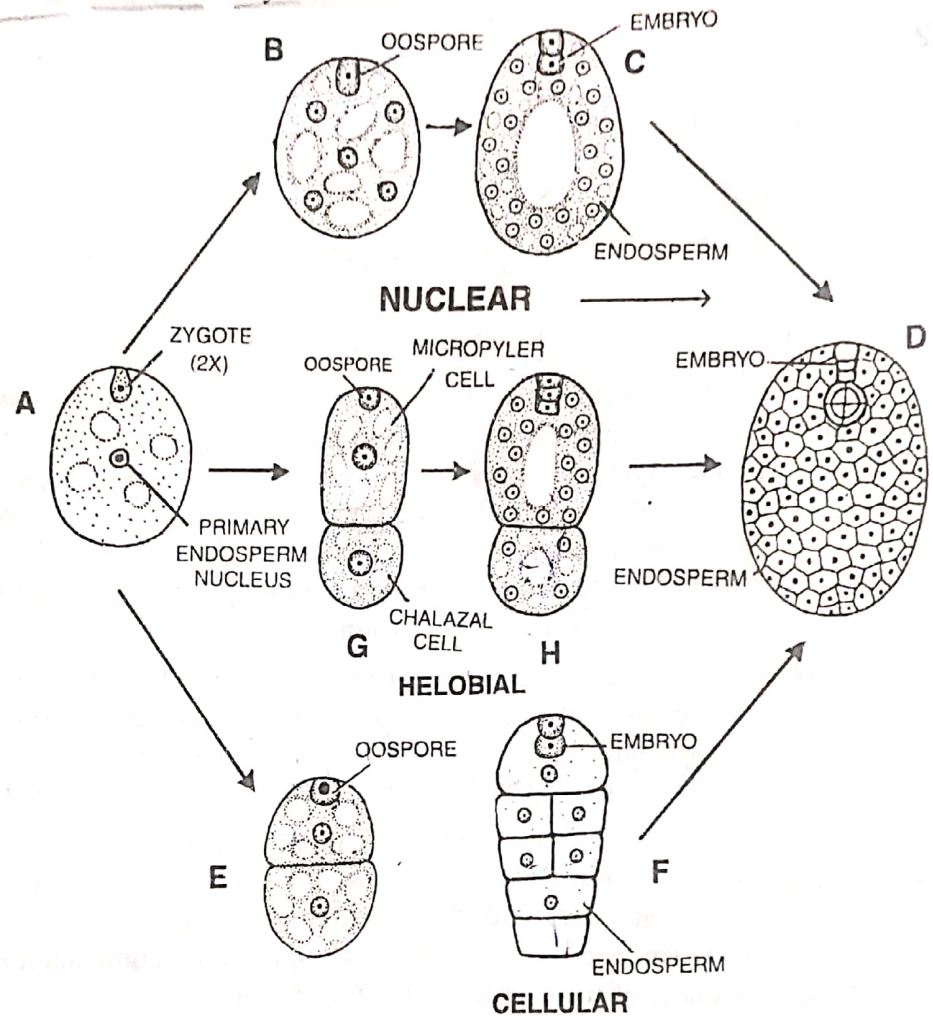


Fig. 7.91. Types of endosperm and their development.

3. **Helobial Endosperm**. The first division of primary endosperm nucleus is followed by transverse cytokinesis to form two unequal cells, larger **micropylar** and small **chalazal**. Further development in both the cells occurs like that of nuclear endosperm *i.e.*, multinucleate stage followed by wall formation. Usually chalazal cell disintegrates after few nuclear divisions, *e.g.*, *Asphodelus, Saxifraga*. It is intermediate between nuclear and cellular type and common in Helobiales. It is found in 19% families, **mostly monocots**.

Fate of Endosperm

During its growth the endosperm crushes the nucellus. It is in turn consumed by growing embryo. The endosperm may persist in the seed when the latter is called **endospermic** or albuminous (*e.g.*, Castor, Cereals, Coconut, Cotton, Sunflower, Palm, Onion, Poppy, Mirabilis, Papaya). In others the endosperm is completely absorbed by the growing embryo and the food reserve gets stored in the cotyledons. Such seeds are called **nonendospermic** or exalbuminous, *e.g.*, Pea, Bean, Groundnut, Almond, Cucurbits, Mustard.

Endosperm is **oily** in *Castor* bean, *Pinus*, Coconut, Cotton; **starchy** in cereal grain; **cellulosic** in Arecanut and *Phytelephas*, Black pepper and **hemicellulosic** in Coffee and Date palm. Coconut has both nuclear endosperm (degenerated; Coconut milk) and cellular endosperm (white oily coconut meat). In some seeds of **dicots**, nucellus is not used up fully. The unused nucellus is filled with food material and lies outside endosperm/embryo sac. It is called perisperm and such seeds as perispermic seeds, e.g., Black pepper, Water lily, Cardamom, Coffee. The perisperm formed by these nucellar cells at chalazal end of the ovule is called **chalazosperm**. It stores fat and starch and is substitute for **endosperm**, e.g., *Cyanastrum*.

Thus, following three tissues serve as source of nutrition to the developing embryo : (i) **Endosperm**, (ii) **Chalazosperm** and (iii) **Perisperm**.

Endosperm becomes irregular, uneven and convoluted in *Areca* (Betel nut/Supari) and *Passiflora* (**ruminate endosperm**). In *Areca*, Date (*Pheonix*) and Vegetable Ivory (*Phytelephas microcarpa*) the endosperm becomes very hard. The cellulosic endosperm of vegetable ivory (ivory palm) is very hard and used in making umbrella handles, buttons, billiard ball, etc.

Histology of Endosperm

The cells of endosperm are isodiametric. They store food. Their exact nature and properties vary from plant to plant. The walls are thin, pits are absent but when endosperm is hemicellulosic, walls are thickened and pitted. Cells have high degree of **polyploidization** during development. Intercellular spaces between cells absent. In cereals, outermost layer of endosperm constitutes aleurone tissue rich in proteins. It secretes hydrolytic enzymes like proteases and amylases. Endosperm shows **no vascular elements** due to lack of differentiation.

The effect of cross pollination on embryo is evident in next generation while effect on endosperm is visible immediately in the seed. Some other characteristis of endosperm.

Xenia and Metaxenia

The immediate effect of foreign pollen/male gamete on visible characters of endosperm is called **xenia**. **Focke** (1881) described the term xenia (*xenia* = foreign), e.g., if the male parent of maize has a yellow endosperm (dominant trait, Y—) and female parent a colourless (white, recessive, yy) endosperm, after fertilization, the endosperm (Yyy) of new seed (YY or Yy) will be yellow. Similarly, if the female plant of sweet corn whose endosperm is sugary is pollinated by pollens of field corn whose endosperm is starchy, the resultant endosperm will be starchy. It means starchy character is being transmitted by the second male gamete which unites with polar nuclei to form endosperm.

The effect of foreign pollen on the development of seed coat or pericarp, *i.e.*, on tissues of seed outside the endosperm or embryo is called **metaxenia**. It is reported in fruits and seed coats of cucurbits, citrus, date palm. **Swingle** (1928) found in the date palm that the period needed for the maturity of fruits and their size varies with the types of pollen grains which bring about the fertilization. The size of fruit and time needed for their ripening varies according to the type of male plant, whose pollen grains are used for fertilization. Thus time of maturity of fruits and their size can be changed according to the type of pollen used in fertilization. Actually embryo/endosperm secretes certain hormones which are diffused into the wall of the seed and fruit to bring a effect. In *Citrus*, colour and flavour of fruit is effected by pollen parent.

Ruminate Endosperm (Fig. 7.92). In Annonaceae, Myristicaceae, Rubiaceae, Palmae and many species of Arecaceae, the endosperm is ridged and furrowed which are very deep and irregular. Such a rough, irregular convoluted endosperm is called ruminate, e.g., Arecanut, Nutmeg. There are two reasons of the formation of ruminate endosperm :

(i) irregular development of the inner surface of seed coat, e.g., *Passiflora calcarata*.

(ii) outer tissues of nucellus and integuments may invaginate into the endosperm to bring about the ruminate condition of endosperm, e.g., *Annona*, Arecanut, *Verbascum*.

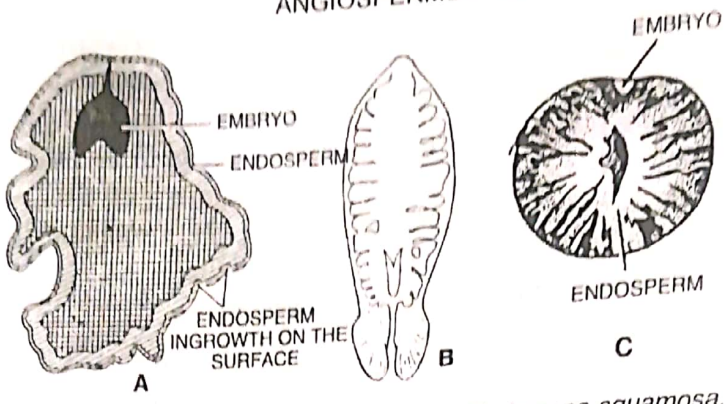


Fig. 7.92. Ruminate (convoluted) endosperm. (A) *Spigelia*, (B) *Annona squamosa*, (C) Betel nut.

Mosaic Endosperm. Occasionally, there is a lack of uniformity in the tissues of endosperm. The patches of two different colours are observed in maize endosperm forming a sort of irregular mosaic pattern, *e.g.*, yellow and white patches are observed irregularly mixed together like a mosaic pattern. **Webber** (1900) reported such patches in endosperm of maize. He gave two reasons of the formation of mosaic endosperm :

(i) After fertilization, second male gamete does not fuse with secondary nucleus. This male nucleus and secondary nucleus divide independently. Thus, the nuclei of two distinct characters get intermixed. This forms variegated endosperm.

(ii) Second male gamete fuses with only one polar nucleus. These both fertilized and unfertilized polar nuclei divide freely and give rise to nuclei of two distinct characters and form a variegated endosperm.

Functions of Endosperm. Endosperm serves as a storage tissue as its cells are ladden up with food absorbed through the placenta. Functionally, **it is nutritive** and supplies food to the developing embryo. The embryo soon stops developing if endosperm is not formed. It provides growth stimulus to embryo. It is used in tissue culture experiment, *e.g.*, coconut, milk.

Morphological Nature of Endosperm

The endosperm is formed as a result of triple fusion. It is, therefore, 3x (triploid). Some times, it may be 5x, 9x to 15 x (maximum in *Arum maculatum*, 24576 x). It is 2x in *Oenothera* and 9x in *Peperomia*, depending upon number of nuclei that fuse to form the endosperm. Whether it is a gametophytic tissue/sporophytic tissue/or a *tissue suigenesis*, it is debatable and has been interpreted variously by different workers as detailed below :

1. Endosperm is a vegetative tissue of the female gametophyte : Workers like **Hofmeister** (1861) and **Strasburger** (1900), Coulter and Chamberlain (1911) have suggested that endosperm is a gametophytic tissue whose development is checked since (i) it stores food for the developing embryo, (ii) formed as a result of free nuclear division like the female gametophyte of gymnosperms.

But it is a new structure developed after gametic union with two polar nuclei. It is triploid and, therefore, cannot be considered endosperm to be a gametophytic tissue.

2. **Haberlandt** (1914) stated that the chief function of endosperm is not that of storage but the secretion of enzymes so that the food stored in the endosperm may be made available to the embryo.

3. **It is a sporophytic tissue.** Sargant (1900) suggested that the endosperm is a sporophytic tissue in which triple fusion results in the formation of a triploid nucleus. Thus endosperm is the **twin of the embryo** and sporophytic in nature. The triple fusion is not regarded as an act of fertilization because (i) the effect of fusion is simply to afford a growth stimulus, (ii) more than a single male and female nucleus are involved in fusion, (iii) the product of triple fusion is not a new individual, but simply a nutritive tissue and (iv) a sporophytic tissue is diploid and not triploid.

4. Endosperm is an **undifferentiated,** entirely new tissue, *i.e.*, **tissue suigeneris.** This is most accepted view about the nature of endosperm. It is triploid (3x) or polyploid and is neither gameto-

phytic nor sporophytic but an undifferentiated special tissue which is stimulated to continue the growth of the female gametophyte that usually stops at eight nucleated stage. **It is primarily a nutritive tissue and should in no way be confused with the endosperm of gymnosperm** which is a true female gametophyte; haploid (x) and developed before fertilization. According to **Brink** and **Cooper** (1947) double fertilization is a device to compensate for the extreme reduction of female gametophyte in angiosperms. Due to an extra amount of chromosomes, it is more active for getting food from nucellus and integuments for embryo. It is special tissue for special physiological function. It has nuclei of both male and female and, therefore, is more vigorously fulfil the need of developing embryo (**Nemec**, 1910)

Endosperm Haustoria

Some of the groups of plants develop haustorial structures from their endosperm which invade nutritive tissues like nucellus of the ovules and absorb the food material by penetrating into them. It is as an extra device to collect the food material. The absorbed food materials are transported to the endosperm tissue to be utilized by the embryo during its growth. Haustoria are common in cellular endosperms, though they are developed in nuclear and helobial endosperm also. These haustoria may develop from micropylar cell (*e.g., Impatiens*) or Chalazal cell (*e.g., Grevillea*) or both (*e.g., Ruellia*; Fig. 7.93).

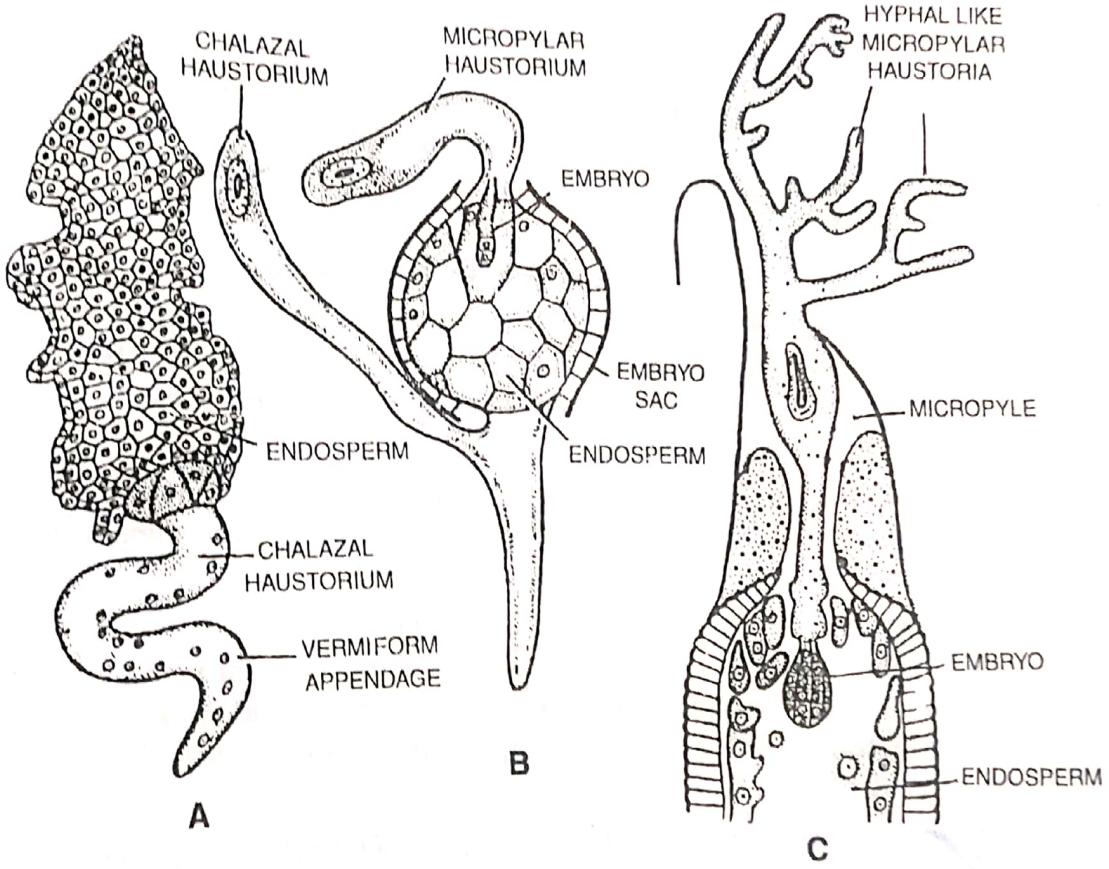


Fig. 7.93. Endosperms haustoria. (A) Chalazal haustorium in *Grevillea robusta*. (B) Micropylar and chalazal haustoria in *Nemrophila*. (C) Micropylar haustorium in *Impatiens roylei*.

1. **Chalazal Haustoria in Nuclear Endosperms.** In *Grevillea robusta* (family Proteaceae) which exhibits free nuclear type of endosperm development, the upper micropylar part of the endosperm is cellular but its lower chalazal part develops into a worm like, tubular and coiled coenocytic structure called vermiform appendage. This appendage, acting as haustorium, invades the chalazal tissue till this tissue is completely obliterated and absorbed. After sometime this appendage becomes divided into several multinucleate partitions giving rise to a secondary endosperm.

Macadamia ternifolia (Proteaceae) is another example where the endosperm is nuclear

type. Like *Grevillea*, in *Macadamia* too, the cell wall formation takes place in upper part because most of the nuclei are aggregated in this portion and the endosperm tissue develops in the upper part of the embryo sac. The lower part of embryo sac, which is having less number of nuclei remains coenocytic and develops much lobed and aggressive haustorial structure. These lobes suck the food material from the basal part of the ovule.

2. **Micropylar Haustoria in Cellular Endosperms.** In *Impatiens roylei* where the development of the endosperm is cellular type, the division of the primary endosperm nucleus is followed by laying down of the first transverse wall giving rise to a small micropylar and a large chalazal chamber. The micropylar chamber then divides transversely into three cells. A very much branched prominent haustorium develops from the upper most cell, the branches of which penetrate even upto the funiculus to absorb food materials.

3. **Micropylar and Chalazal Haustoria in Cellular Endosperm.** In many members of Acanthaceae (*e.g., Ruellia, Acanthus, Nemrophila*), the endosperm develops both micropylar and chalazal haustoria. After the first nuclear division of the primary endosperm nucleus and a cell wall formation, the two cells, *i.e.*, micropylar cell and chalazal cell are formed. The chalazal cell develops a small chalazal haustorium which remains coenocytic. A transverse division takes place in the micropylar cell which results in two cells, out of which the upper cell develops the micropylar haustorium.

4. **Micropylar Haustoria in Helobial Endosperm.** In *Monochoria* (family Pontederiaceae) the development of endosperm is helobial type. The chalazal chamber, with less number of nuclei, remains smaller than the micropylar chamber with more nuclei. Later, two tubular growths develop from the two sides of the micropylar chamber, embracing the chalazal chamber on its each side. These outgrowths or lateral haustoria grow in the direction of chalazal tissue, penetrate into it and function as aggressive absorptive organs.

Endosperm–Embryo Relationship

As a result of triple fusion, endosperm is formed in all seeds of angiosperms except three families. During embryo development, in some seeds, endosperm is consumed (*e.g.*, exalbuminous seeds) or not consumed completely (*e.g.*, albuminous seeds). In some seeds, nucellus persists to form perisperm (perispermic seeds).

It is recorded that the development of endosperm occurs prior to the development of embryo. It provides food to developing embryo as well as some growth stimulus to the embryo. Embryo soon stops developing if endosperm is not formed.

Thomas (1900), Nemec (1910), Brink and Cooper (1947) found that endosperm nuclei have both male and female contents and, therefore, more vigorous and fulfil the need of developing embryo. Rao found some relationship between endosperm type and the growth of embryo. In free nuclear endosperm, growth of embryo is rapid. This growth is slow when endosperm is of cellular type.

(B) EMBRYOGENESIS (EMBRYO FORMATION)

Study of structure and development of embryo from zygote is called embryogenesis. After syngamy, zygote (oospore) is formed. This zygote undergoes a period of rest of few hours to years. This resting period is short in cellular endospermic seeds. In general, development of zygote occurs after the development of endosperm.

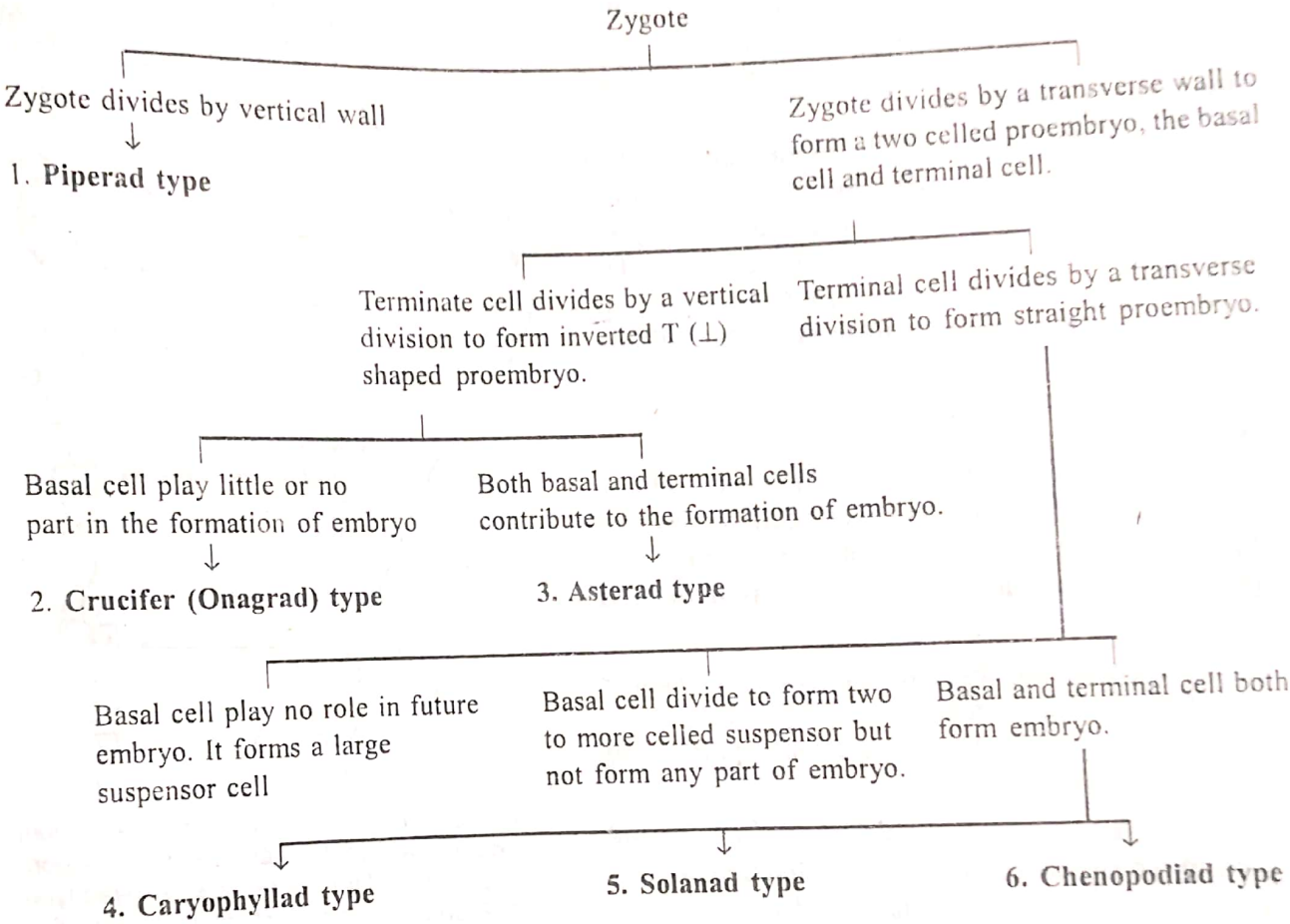
Development of embryo is **meroblastic**, *i.e.*, only a part of zygote develops into embryo. Unlike the gymnosperms, each division of embryo development is followed by wall formation.

In the earlier stages of development (upto 8 celled octant stage), the development of embryo is similar in both monocots and dicots. However, the further development is markedly different in two groups. Organogenesis set in at 16 – 32 celled stage of embryo. The first organ to be initiated is the scutellum/cotyledon.

Dicot Embryo

Usually the first division of zygote is transverse. It forms a two celled proembryo. Of the two cells thus formed, upper one, towards the antipodal/chalazal end is smaller called **embryo cell or terminal cell** and the other towards micropylar end is larger called **basal cell or suspensor cell**. The basal cell does not contribute towards the development of future embryo. It simply forms suspensor.

Maheshwari (1950), Johansen (1950) have recognised following six types of development of embryos among the angiosperms according to the plane of divisions and number of cells taking part in the development of embryo. This system of Johansen is based on Schnarf's system who recognised five types (Fig. 7.94).



Of all the six types, **crucifer type** is considered as the **typical** mode of **embryo development in dicots.**

Laws of Embryogenesis. Soueges (1931) and Johansen (1950) recognised that embryo development follows certain laws which are specific to every species. They have proposed 5 laws applicable to all the species.

1. **Law of Parsimony** (Law of economy). It states that only as many cells are produced by the embryo as needed.
2. **Law of Origin.** This law states that sequence of cell formation is very regular and predetermined in each species.
3. **Law of Numbers.** The number of cells produced by different cell generations varies with the species and depends upon the rate of divisions of different cells.
4. **Law of Disposition.** It states that the cells are constituted by divisions, indetermined directions and occupy the positions in accordance with the part they must play.

5. **Law of Destination.** According to this law, in any given species, the cells of the proembryo give rise to clearly determined parts and always to the same parts of the embryo.

Development of a Typical Crucifer (Onagrad) Type of Dicot Embryo

The most common type of development of embryo in dicots is reported in Shepherd's purse (*Capsella bursa-pastoris*, Cruciferae; Hanstein, 1870, Soueges 1914, 1919; Fig. 7.94).

(a) The zygote (oospore) elongates and divides by a transverse wall into **two** unequal cells (Schulz and Jensen, 1969). The larger lower, **basal cell** towards micropylar end called **suspensor cell (Cb)** and other smaller, upper towards antipodal/chalazal end is called **terminal cell (embryo cell; Ca).**

(b) The basal cell divides by a single transverse division while the terminal cell by a vertical (longitudinal) division to form a **inverted T (⊥) shaped four celled proembryo.**

(c) Both the basal cells divide transversely a few times to form a 6 – 10 celled filamentous structure called **suspensor.** It pushes the developing embryo in the endosperm where food is available. The upper most cell of the suspensor towards the micropylar end gets swollen to form **vesicular cell** and acts as a **haustorium.** The haustorium has wall ingrowths. The last cell of the suspensor at the end adjacent to the embryo cell is called **hypophysis.** It divides transversely into two cells : (i) outer cell forming root cap and epiblema, (ii) inner cell forming root tip and cortex and pith, *i.e.*, **hypophysis cell of suspensor forms all parts of radicle except hypocotyl** (Fig. 7.95).

Suspensor thus does three functions : (i) pushing of the embryo deep into endosperm for regular availability of food, (ii) formation of radicle and (iii) absorption of food.

(d) The two embryo cells, of four celled proembryo divide by one vertical division at right angle to first one and one transverse division to form an eight celled globular proembryo (octant stage).

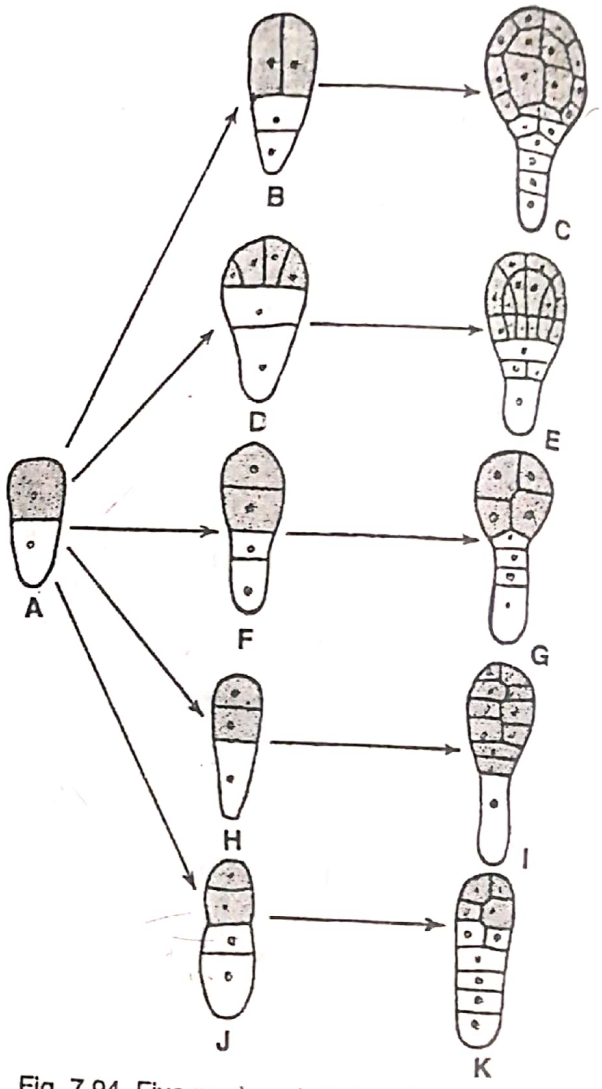


Fig. 7.94. Five modes of embryo development in dicots. (A) Zygote after transverse division. (A, B, C) Crucifer type. (A, D, E) Asterad type. (A, F, G) Solanad type. (A, H, I) Caryophyllad type. (A, J, K) Chenopodiad type.

These eight cells are arranged in two tiers— **epibasal** (terminal) and **hypobasal** (near the suspensor). The two opposite cells of epibasal tier eventually form the two cotyledons and the plumule. The hypobasal cells produce the **hypocotyl** except its tip.

The eight embryonic cells or octants divide **periclinally** to produce an outer layer of **protoderm** or **dermatogen.** The inner cells further divide periclinally to form **inner procambium** (plerome) and **middle ground meristem** (periblem). Protoderm forms epidermis, procambium gives rise to **stele** or vascular strand and ground meristem produces cortex and pith. The development of embryo upto octant stage is quite similar in dicots and monocots.

Initially the embryo is **globular** and undifferentiated. Early embryo with radial symmetry is called **proembryo.** It is transformed into embryo with the development of radicle, plumule and cotyledon. Two **cotyledons** with a faint plumule in the centre are developed from the two opposite lateral cells of embryo. At this time the embryo becomes **heart-shaped.** The rate of growth of the cotyledons is very high

so that they elongate while the plumule remains as a small mound of undifferentiated tissue (**Torpedo** strape). In *Capsella bursa–pastoris*, the elongating cotyledons curve due to the curving of the ovule itself.

A typical dicot embryo consists of an embryo axis with two lateral cotyledons. The part of embryo axis above the region of point of attachment of cotyledons is called *epicotyl* and the part on below radicle is called *hypocotyl*. At the terminal end of epicotyl is plumule (future shoot). The hypocotyl bears radicle (future root) at its tip. With the growth of embryo, the ovule enlarges. Its integuments ultimately become hard to form protective converings. Now the embryo undergoes rest and the ovule gets transformed into seed. In some plants the embryo remains in the globular or spherical form even at the time of seed shedding without showing any distinction of plumule, radicle and cotyledons, *e.g.*, Orchids.

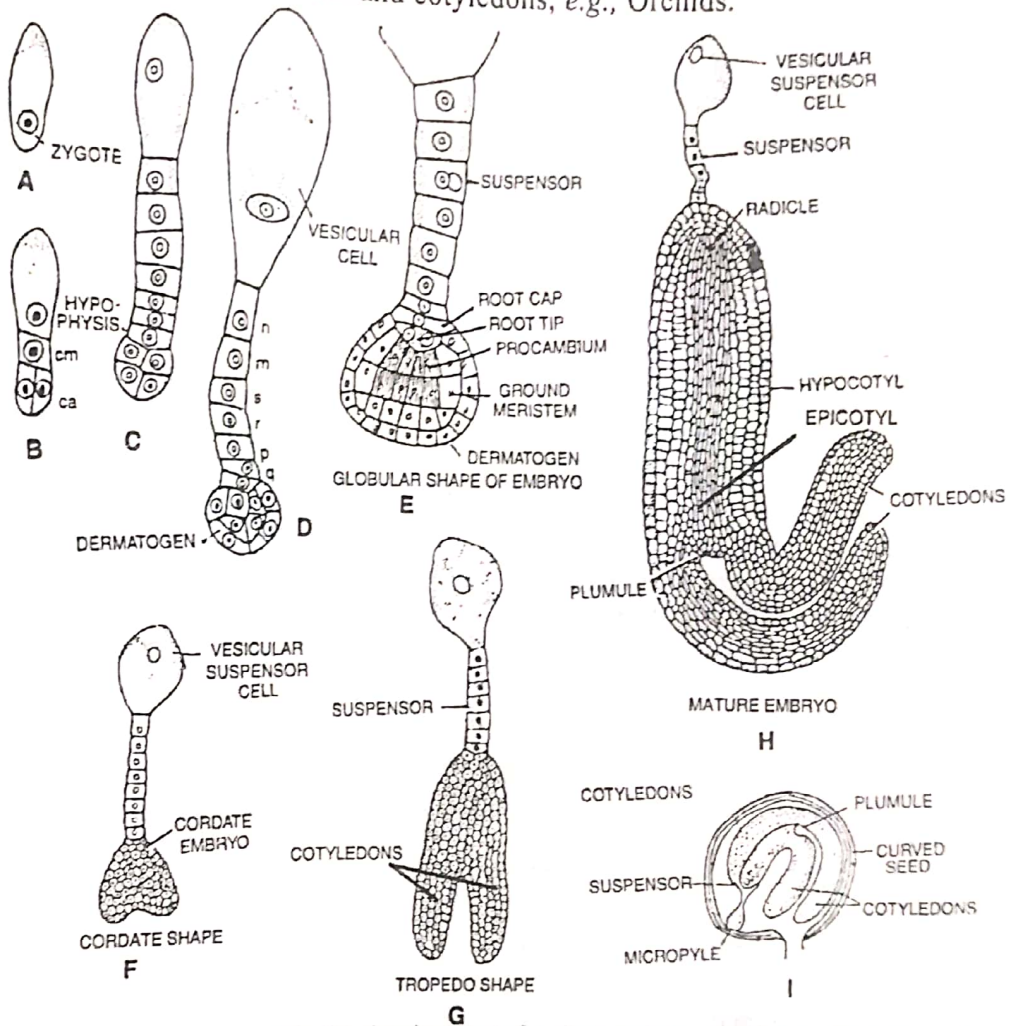


Fig. 7.95. (A-G) Successive stages in the development of a dicot embryo. (A) Zygote (B) ⊥ Shape four celled proembryo (C) Eight Celllled embryo (D) Muticellular embryo. (E) Globular (F) Heart-shaped (Cordate) embryo. (G) Torpedo Shape of embryo (H) Mature embryo(I) Horse shoe shaped embryo in *Capsella bursa pestoris.*

Development of Embryo in Monocots

There is no monocot plant whose embryogeny can be taken as a typical one for type study. However, caryophyllad type of development of embryo is the representative of the most of families of monocots.

In monocots, the embryonal axis has a single terminal cotyledon. The epicotyl/plumule is lateral due to slow growth of epicotyl and fast growth of cotyledons.

The embryogeny of *Sagittaria sagittifolia* is described below (Fig. 7.96 B).

The zygote first divides by a transverse wall into a terminal cell (*t*) and a basal cell (*b*). The basal cell, situated towards the micropylar end, does not divide further, but becomes much

enlarged to form a conspicuous **vasicular structure** forming a part of suspensor. The terminal cell forms whole of embryo as well as remaining part of suspensor. It undergoes a transverse division to form two cells t' and t''. Now the **proembryo** attains a linear superposed **three celled stage** (t', t'', b). During development, t′ forms single cotyledon and t″ forms epicotyl, hypocotyl, radicle and suspensor. **This makes the cotyledon apical and plumule lateral.** The lower cell (t') of this stage divides first by a longitudinal wall, followed by another longitudinal wall, perpendicular to the first. These resultant four cells now divide by a transverse wall. Thus, eight cells are produced in two tiers t_1 and t_2 of four cells each. With further growth and divisions, these cells form dermatogen, cotyledon, plerome and periblem. Now the middle cell (t'') of the three called stage of the proembryo divides by a transverse wall into two cells (a and h). Out of these two cells formed, the lower cell (a) divides vertically twice to give rise to the epicotyl and plumule. The upper cell (h), after undergoing few transverse divisions forms a filament of four cells : (i) h_1— it forms root tip, (ii) h_2— form periblem and a part of root cap, (iii) h_3— forms part of root cap and hypocotyl and (iv) h_4— forms 3 – 6 celled suspensor.

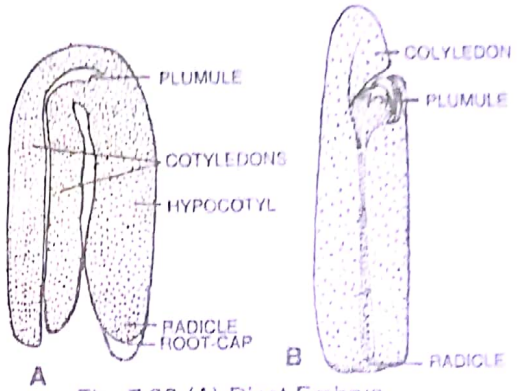


Fig. 7.96 (A) Dicot Embryo, (B) Monocot Embryo.

Differences between Monocot Embryo and Dicot Embryo

Monocot Embryo	*Dicot Embryo*
1. Cotyledon is one and terminal.	1. Cotyledons two, lateral.
2. Plumule is lateral.	2. Plumule is apical.
3. Suspensor is formed by both basal & terminal cell.	3. Suspensor is formed by basal cell alone.
4. Radicle is formed by one of the cells of suspensor.	4. Radicle is formed by embryo cell.
5. Proembryo is three celled and linear.	5. Proembryo is four celled and inverted ⊥ shaped.
6. First division of terminal cell is transverse.	6. It may be longitudinal or transverse.
7. The number of cells used in the formation of cotyledons is 4, 3 or 2.	7. The cotyledons developed from two opposite cells of the four celled epibasal tier of embryo.
8. Terminal cell forms the whole of the embryo.	8. Terminal cell produces embryo except the radicle.

The periclinal walls appear in the right cells situated at the distal end of the developing embryo and dermatogen is formed. The differentiation of the **periblem** and **plerome** follows later. This entire region by further growth and development develops a single **cotyledon**. Finally, the **shoot apex** originates laterally in a depression situated at some place in between the axis of embryo and the base of cotyledons. Thus, it is lateral in position in contrast of the shoot apex of the dicotyledons in which it is terminal. The radicle, plumule and suspensor, all develop from the terminal cell and the basal cell does not divide at all. Suspensor is one celled in wheat. It is absent in Arecaceae (Fig. 7.96).

In grasses/cereals, the development and structure of embryo is different from other monocots. The single cotyledon is large shield shaped called **scutellum**. The embryo axis below the attachment of scutellum is called hypocotyl which at its tip bears radicle. Radicle has root apex and root cap covered by a solid envelope called **coleorhiza**. Embryo axis above the level of scutellum is called epicotyl. It bears plumule (shoot apex) and few leaf primordia which is enclosed in a hollow foliar covering called **coleoptile**. The remnants of second cotyledon is called epiblast. Both coleoptile and coleorhiza are derived from scutellum.

Nutrition of Embryo.

Embryo takes its nourishment from following sources.

1. **Endosperm.** It is a nutritive tissue that provides nourishment to the developing embryo. It

also develops haustoria to transfer food from ovular tissue to the embryo. (2) **Perisperm.** It is the part of the nucellus left in the seed and becomes rich in food eg Black pepper seeds. (3) **chalosperm.** It is the part of nucellus left in the seed at chalazal end where it divides to form a fat and starch rich tissue. (4) **Haustoria.** Haustoria help in the absorption of food from ovule and transfer it to the developing embryo *e.g.*, Suspensor haustoria, antipodal haustoria, embryo sac haustoria, endosperm haustoria. (5) **Pseudo embryo sac.** In family Podostemonaceae, pseudoembryo sac is developed by the breakdon of nucellus. It is used up by developing embryo.

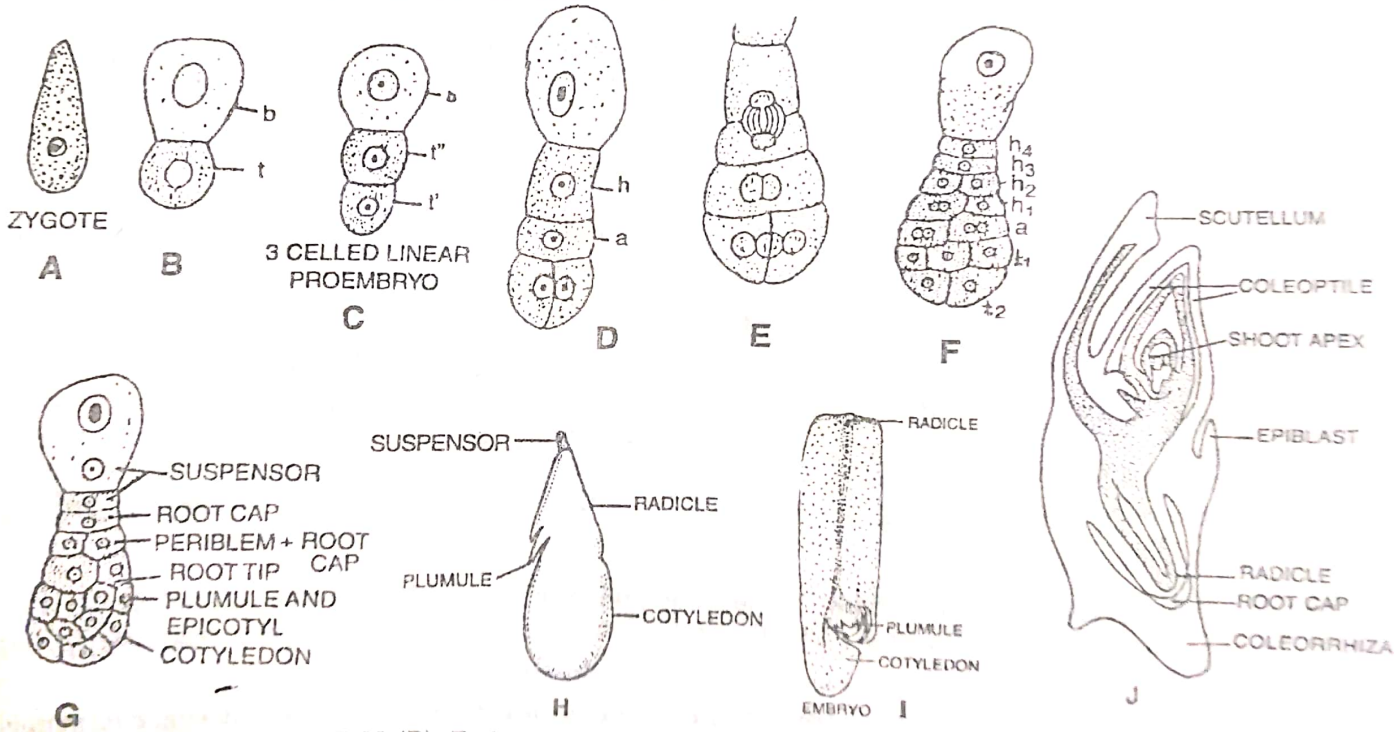


Fig. 7.96 (B). Embryo development in a monocot *Sagittaria*.
(t) Terminal cell, (b) Basal cell, *t'* and *t''*, formed by terminal cell. *a* and *h* formed by *t'* . (I) Mature embryo of a monocot (j) Mature monocot embryo of a grass/cereal.