UNIT 5 EMBRYOGENESIS

Structure

5.1 INTRODUCTION

In the previous units 1, 2, 3 you have studied all the aspects upto the fertilization. In this unit you are going to study embryogenesis in detail. The process of double fertilization culminates in the fusion of one of the male gametes (discharged by the pollen tube) with the egg and of the second male gamete with the fusion nucleus in the central cell. As you have already studied in units 3 and 4, the fusion of a male gamete nucleus with the polar nuclei results in transformation of the central cell into the primary endosperm cell. It is this cell which gives rise to the nutritive tissue called the endosperm in the seed. The fertilized egg, (zygote) develops into the embryo and is the forerunner of the sporophyte. The embryo through embryogenesis develops into mature embryo.

Objectives

After studying this unit you should be able to:

- explain the stages through which an embryo is formed starting from zygote,
- distinguish between a monocotyledonous and dicotyledonous embryo,
- describe how the embryo derives its nutrition.

5.2 ZYGOTE

The fertilized egg or zygote is situated at the micropylar end/pole of the embryo sac, its basal (micropylar) end is attached to the embryo sac wall and apical (chalazal) part projects into the central cell. The zygote usually undergoes a period of rest during which it shrinks. This resting period of the zygote varies with different species and is to some extent dependent on environmental conditions e.g. in *Theobroma cacao* the zygote divides 14 to 15 days after fertilization, in *Oryza sativa* zygote divides about 6 hours after fertilization. A complete cell wall is formed around the zygote. (You would recall that the egg has only plasmalemma and no wall at its apical part). A TEM picture shows that zygote cytoplasm show a more polarized appearance and that is the two poles appear different, micropylar part is vacuolate and chalazal part with a prominent nucleus (Fig. 5.1).

Fig. 5.1: *Gossypium hirsutum*, zygote after four hour of fertilization—highly polarized zygote; clustering of plastids and mitochondria around the nucleus at the chalazal pole. (After Jansen 1968)

A marked increase occur in the density of cytoplasmic organelles such as mitochondria, dictyosomes and plastids. Endoplasmic reticulum becomes more extensive and the density of ribosomes and polysomes increases indicating intense metabolic activity. Now we will study early embryogenesis in embryo.

5.3 EARLY EMBRYOGENESIS

In a majority of angiosperms the zygote divides by a transverse wall (Fig. 5.2) resulting in a smaller apical cell (usually designated as *ca*) and a relatively large basal cell (designated *cb*). The division of the zygote is exceptionally veritcal or oblique, (as in members of the Loranthaceae and the Piperaceae). Beginning from the 2-celled stage up to the initiation of cotyledons the young sporophyte is commonly called the proembryo. In the 2-celled proembryo the basal cell *cb* usually divides transversely to form two cells *m* and *ci* (Fig. 5.3A). The apical cell *ca* may divide vertically or transversely, so that the 4-celled proembryo has either a linear (all four cells in a row) (Fig. 5.3B) or a T-shaped (Fig. 5.3C) configuration. In the linear proembryo the two daughter cells of *ca* (l & l') may undergo two vertical divisions at right angles to each other to give rise to an octant having two superposed tiers (l & l') of four cells each (Fig. 5.3D).

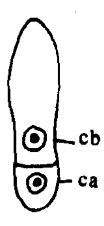

Fig. 5.2: Division of zygote by transverse wall.

In the T-shaped proembryo, a vertical division at right angles to the first vertical division in the apical cell can produce a quadrant *q*. (Fig. 5.3E). A transverse division in each cell can then produce an octant similar to the one produced by the linear 4-celled proembryo. (Fig. 5.3F). The T-shaped proembryo can also give rise to an octant by vertical tangential divisions in the four cells of tier *q* so that all the eight cells are included in the same tier (Fig. 5.3G).

The cells derived from *ci*, *m* and *q* divide further and differentiate to form the different parts of the mature embryo. The early development of the proembryo is similar in both monocotyledons and dicotyledons. After the octant stage, the destiny of the various cells of the proembryo differs in these two major groups. Because of these later

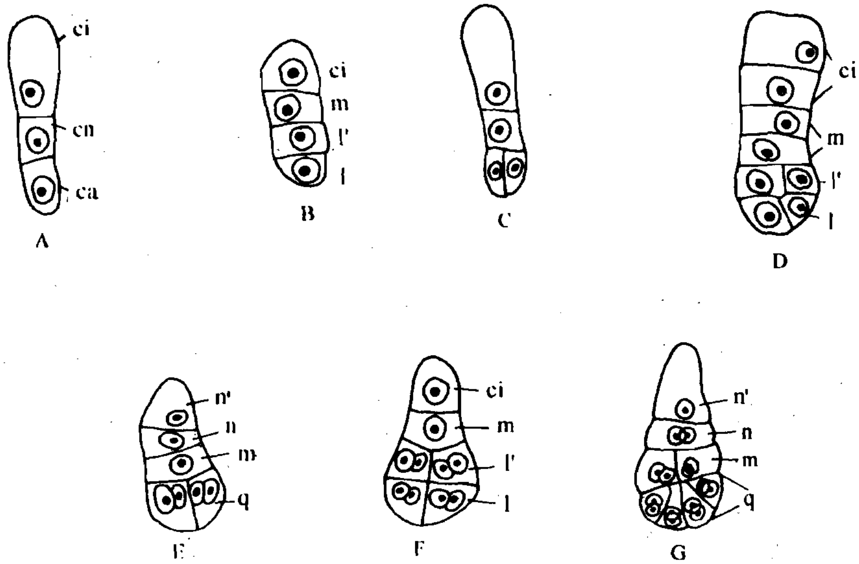

Fig. 5.3: Division of angiospermic embryo.

differences the mature dicot embryo possess an apical shoot apex and two lateral cotyledons (Fig. 5.4), whereas the monocot embryo has only one cotyledon and a somewhat laterally placed shoot apex (Fig. 5.5). The structure of the dicot and the monocot embryos would be described in greater detail later in this unit after giving you a general account of the development of the proembryo.

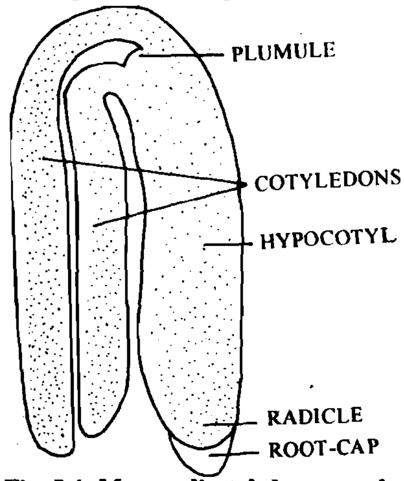

Fig. 5.4: Mature dicotyledonous embryo.

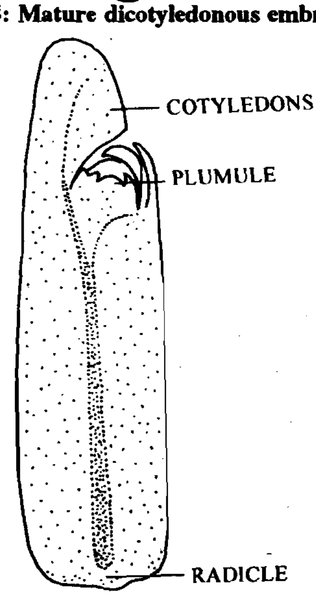

Fig. 5.5: Mature monocotyledonous embryo.

Types of Embryogeny

On the basis of the plane of division of the zygote and of the cells of the 2-celled proembryo, and also taking into account the relative contributions of the cells of the 4-celled proembryo to the mature embryo, six cheif types of embryogeny have been recognised (Johansen, 1950; Maheshwari, 1950).

A. Division of the zygote is vertical — Piperad type (e.g., Loranthaceae, Piperaceae).

AA. Division of the zygote is transverse.

B. Apical cell of the 2-celled proembryo divides vertically to form a T-shaped, 4-celled proembryo.

C. Basal cell plays no role at all or only an insignificant role in subsequent development of the proembryo—(Crucifer or Onagrad type (e.g., Ranunculaceae, Brassicaceae).

CC. Basal cell and apical cell both contribute to the development of the embryo — Asterad type (e.g., Asteraceae, Violaceae).

BB. Apical cell of 2-celled proembryo divides transversely so that the 4-celled proembryo is usually linear.

D. Basal cell does not participate or only contributes a little to development of embryo proper.

E. Basal cell usually forms a suspensor — Solanad type (e.g., Solanaceae, Linaceae).

EE. Basal does not divide further and the suspensor, if present, is derived from the apical cell — Caryophyllad type (e.g., Caryophyllaceae, Crassulaceae).

DD. Basal cell and apical cell both divide and contribute to formation of the embryo — Chenopodiad type (Chenopodiaceae, Boraginaceae).

SAQ 1

State whether the following statements are true or false. Write either T or F in the boxes provided.

a) Zygote usually has a densely-cytoplasmic basal (micropylar) part and a vacuolate apical (chalazal) part. [ ]

b) Zygote has a complete cell wall around it. [ ]

c) Division of the zygote is nearly always vertical or oblique. [ ]

d) Early development of the proembryo is similar in monocots and dicots. [ ]

e) In Caryophyllad type of embryogeny the basal cell forms a well developed suspensor. [ ]

5.4 HISTOGENESIS AND ORGANOGENESIS

After the octant stage numerous cell divisions occur in various planes. The proembryo become globular or bulb-shaped. As a general rule, some tangential divisions occur in the cells of the octant so that three cells layers are differentiated—the outer **dermatogen** which later forms the epidermal covering, middle **periblem** which gives rise to the cortex of the stem and root, and inner **plerome** that is responsible for the vascular tissue and pith. Such a process of differentiation of cell layers is termed **histogenesis**.

Organogenesis or differentiation of the initials of various organs begins in the globular proembryo in such a way that cotyledons (which form the first leaves), **epiphysis** (which gives rise to the stem apex) and **hypophysis** (that forms the root cortex and cap) are produced. Since organogenesis differs in dicots and monocots, we may discuss this aspect separately for the two groups of angiosperms with the help of suitable examples.

5.5 DICOTYLEDONOUS EMBRYO

The development of a dicotyledonous embryo may be illustrated with the help of the familiar classical example of *Capsella bursa-pastoris*. Embryogeny in this species conforms to Crucifer or Onagrad type, in the classification that you have just studied. Division of the zygote is transverse resulting in a basal cell **cb** and a terminal cell **ca** (Fig. 5.6 A, B). Basal cell divides transversely, whereas the terminal cell divides longitudinally. The 4-celled proembryo, thus, has an inverted T-shaped appearance (Fig. 5.6 C). Each of the two terminal cells next divides by a vertical wall oriented at right angle to the first, forming a quadrant (Fig. 5.6 D). The quadrant cells divide transversely to form an octant (Fig. 5.6 E). By carefully tracing the course of subsequent development, it is possible to conclude that the derivatives of the lower four cells of the octant give rise to the stem tip and cotyledons whereas the cells derived from the upper four cells of the octant form the hypocotyl. All the eight cells divide periclinally (Fig. 5.6 F).The outer derivatives form the dermatogen, while the inner undergo further divisions (Fig. 5.6 G) to form the cortical, vascular and pith regions. At this stage, the proembryo may be said to be in globular stage of development. While these developments are taking place in the terminal cell, the derivatives of *ci* and some cells derived from the intermediate cell *m* of the 4-celled proembryo divide to form a row of 6 to 10 suspensor cells (Fig. 5.6G). The upper most cell *v* of the suspensor becomes vesicular and serves a haustorial function. Some derivates of *m* contribute to the suspensor. The lower most cell which is placed between the suspensor and the embryonal mass is generally referred to as hypophysis or *h* (Fig. 5.6 H).

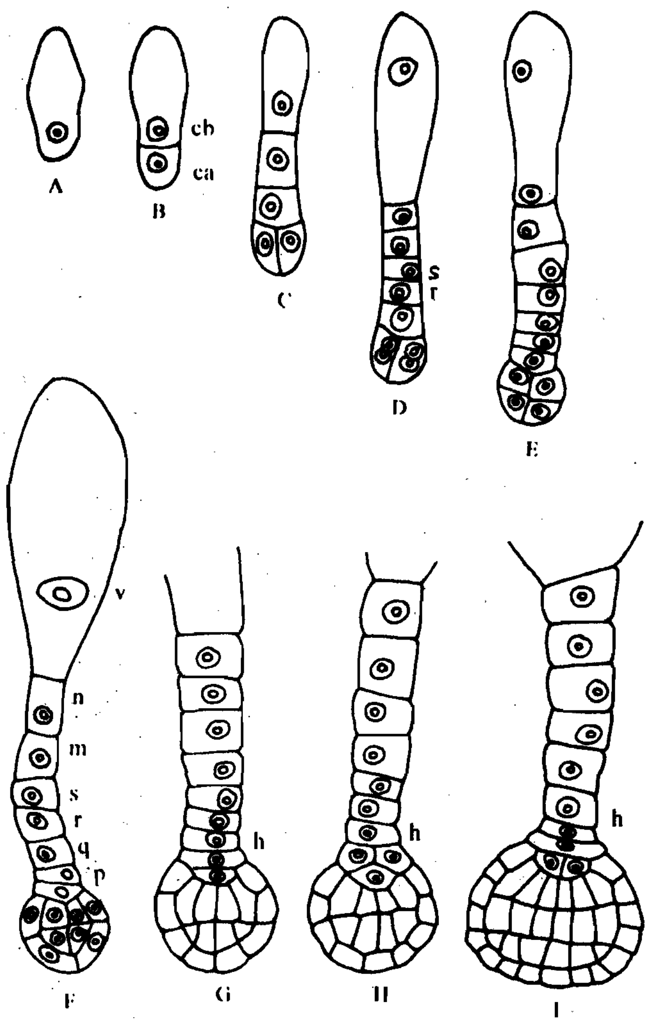

Fig. 5.6: Embryo development in *Capsella bursa -pastoris*.

This cell *h* undergoes a transverse division followed by two longitudinal divisions at right angles to each other in both the derivates. This results in a group of eight cells, of which the inner four give rise to the initials of the root cortex and the outer (toward the suspensor) four form the root cap and root epidermis.

The globular proembryo undergoes further cell multiplication, especially at the two points which are destined to form the cotyledons. At the stage when cotyledons are initiated the embryo is cordate or heart-shaped (Fig. 5.7A). Between the two cotyledons i.e., at the tip of the embryonal a wedge shaped group of cells is cut off which represents region the epiphysis or forerunner of the shoot tip. The hypocotyl and the cotyledons elongate so that the embryo become torpedo-shaped. (Fig. 5.7 B). During further development in *Capsella* the elongating cotyledons become curved like a horse shoe (Fig. 5.7C). However, in a majority of dicotyledons the mature embryo is straight.

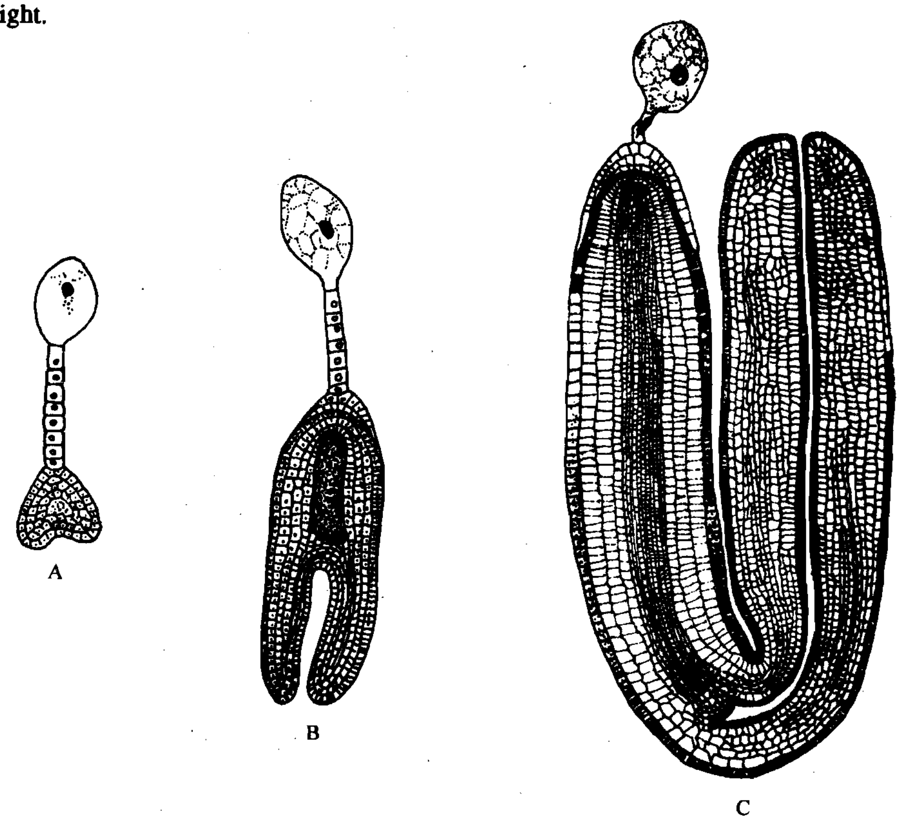

Fig. 5.7: Later stages of development of *Capsella embryo*.

5.6 MONOCOTYLEDONOUS EMBRYO

The early development of the proembryo in monocots follows the same pattern as in the dicots. However, at the time of differentiation in the globular proembryo certain fundamental differences arise. In monocots one half of the terminal cell and its derivatives have retarded growth, whereas the other half grows rapidly to form one cotyledon. As a result of this assymetric growth, in later stages the stem tip, which is also derived from the terminal cell, appears to be lateral in position.

The major differences between the dicot and the monocot embryos arise due to disparity in the number and position of the cells of the terminal quadrant of the proembryo which contribute to the formation of the cotyledonary and epicotyl regions. In the dicotyledons derivatives of the two opposite cells of the terminal quadrant give rise to the two cotyledons (Fig. 5.8 A, B). Among the monocotyledons the number of cells of the quadrant that contribute to the cotyledons varies (Fig. 5.8 C, D, E).

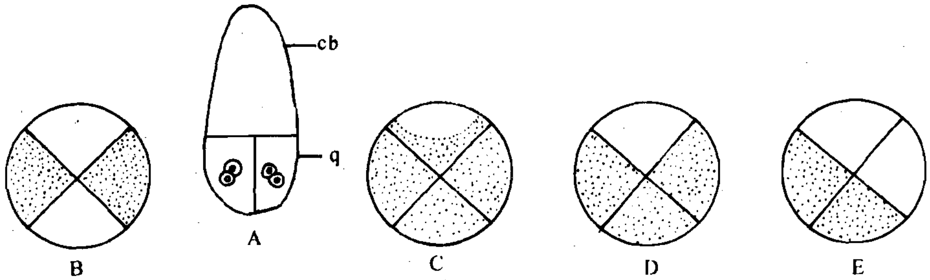

Fig. 5.8: Derivation of cotyledons in monocotyledonous and dicotyledonous. A. Quadrant proembryo. B. Development in dicotyledons. C-E. Development in various monocotyledonous taxa.

5.7 MATURE EMBRYO

A typical dicotyledonous embryo, as seen in a median longitudinal section, consists of an embryonal axis having two broad cotyledons. The portion of embryonal axis above the level of cotyledons is termed epicotyl which terminates in the plumule or stem tip. The cylindrical portion below the level of cotyledons is called the hypocotyl which terminates at the lower end in the radicle or root tip. The root meristem is covered by a well defined root cap.

The embryo of monocotyledons, possesses only one cotyledon. The grass embryos is highly specialized and has received a great deal of attention. It has a single cotyledon in the form of scutellum, which appears to be laterally attached to the embryonal axis (Fig. 5.9). At its lower end the embryonal axis has the radicle and root cap, enclosed in an undifferentiated part of the embryo called *coleorhiza*. On one side the *coleorhiza* is given out a small outgrowth called the epiblast. The portion of embryonal axis above the level of attachment of the scutellum is termed epicotyl. It has a shoot apex with some leaf primordia, enclosed in a hollow foliar structure called the coleoptile.

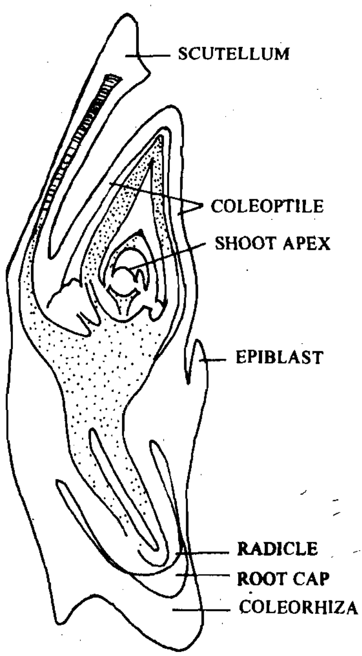

Fig. 5.9: Median longitudinal section of mature embryo of *Triticum*.

As you may be already aware, the embryo remains embedded in the endosperm in the seed, which in turn is enclosed in the fruit. The seed is dispersed by the plant and serves as a unit of propagation. The seed can also perennate in the soil till appropriate conditions are available for its germination. On germination of the seed, the cotyledons expand and appear leaf-like, the epicotyl grows with the help of the meristem at its tip to form the stem axis, and the radicle or root meristem forms the primary root.

SAQ 2

In the following statements, tick the correct ones (√) and cross the wrong ones (X):

a) Development of early embryo takes place in the same, precise manner in all the dicotyledons. [ ]

b) Dermatogen divides anticlinally to form the epidermal cells of the embryo. [ ]

c) Initials of the root cap and root cortex are derived from hypophysis. [ ]

d) In the monocotyledons embryo, the stem tip is not derived from the terminal cell. [ ]

e) The epicotyl terminates in the plumule which forms the primary root. [ ]

5.8 MODIFICATIONS OF SUSPENSOR

In the early part of this unit much of the attention was focussed on the development of the terminal part of the proembryo which produces the embryo proper. However, you would recall that the basal cell also divides contributes to the formation of a suspensor. The suspensor grows relatively fast in the early stages and usually attains the maximum size at globular or the heart-shaped stage of the embryo. Later it degenerates and at maturity only vestiges of the suspensor will be seen attached to the embryo.

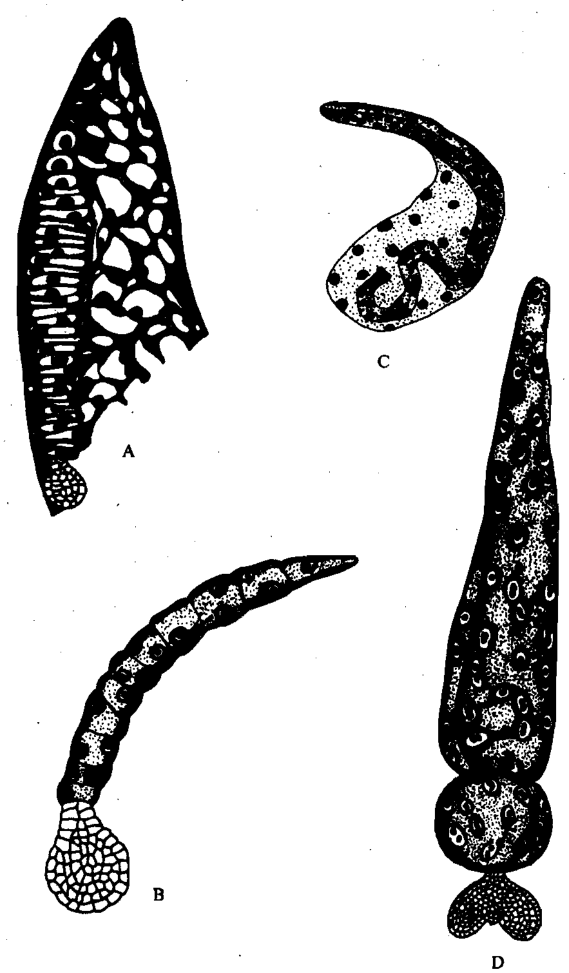

Fig. 5.10: Various modifications of suspensor cells.

It was believed earlier that the function of the suspensor was merely to hold the embryo proper and push it into the nutritionally rich endosperm. However, detailed investigations cytochemistry and ultrastructure of the suspensor have shown a more active role for the suspensor. The diversity in size, shape, longevity and cytological characteristics of suspensor observed among different taxa relate to the mechanism of function of suspensor in the nutrition of the embryo.

In some flowering plants (e.g., *Viola* and *Tilia*) there is no suspensor and in many others (e.g., *Euphorbia* and *Bryonia)* the suspensor is highly reduced. It is obvious that in such plants the suspensor could play little or no role in the nutrition of the embryo. However, in several families (e.g., Brassicaceae and Loranthaceae) a long filamentous suspensor is present. In the Fabaceae the suspensor displays considerable diversity. While in some legumes the suspensor is poorly developed in others there is a uniseriate (Fig. 5.10 A) or biseriate (Fig. 5.10 B) filamentous suspensor. In *Cytisus laburnum* the cells of the suspensor are clustered like a bunch of grapes (Fig. 5.10 C) and in *Pisum sativum* the suspensor is composed of four large, multinucleate cells (Fig.5.10 D). Suspensors with such large, multinucleate cells are considered to be haustorial as they are believed to derive nutrition from the surrounding cells in an aggressive manner.

You have already read that the Orchidaceae, Podostemaceae and Trapaceae lack endosperm formation. In the absence of endosperm, the embryo usually develops an elaborate suspensor haustorium. In the Orchidaceae the suspensor shows various modifications. It may be:

a) single celled, vesicular or sac-like (e.g., *Dendrobium*, Fig. 5.11 A);

b) a uniseriate filament of 5-10 cells which form haustorial branches in the placental tissue (e.g., *Ophrys*, Fig. 5.11 B);

c) like a bunch of grapes (e.g., *Epidendrum*, Fig. 5.11 C);

d) consisting of eight cells, formed by three vertical divisions in the suspensor initial, elongating downward and enveloping almost half of the embryo (e.g., *Vanda*; Fig. 5.11 D);

e) an irregular mass of 6-10 cells, of which some cells situated toward the micropylar end elongate and form tubular structures (e.g., *Cymbidium*, Fig.5.11 E).

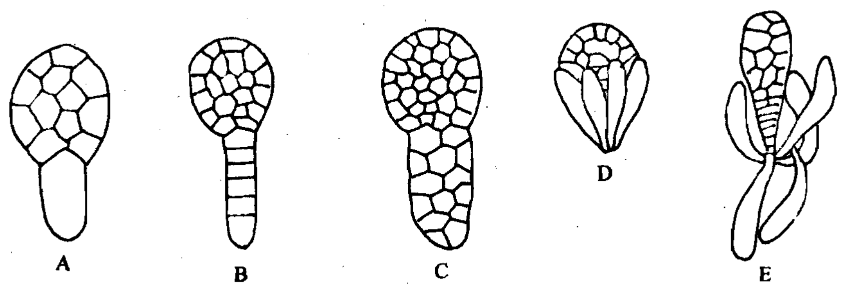

Fig.5.11: Some Types of suspensor cells found in orchids.

In the Podostemaceae (*Dicraea*; Fig. 5.12), the basal cell of the 2-celled proembryo enlarges and contains two hypertrophied nuclei. As the proembryo grows, the basal cell gives out a number of thin-walled haustorial branches which grow in the space between the two integuments of the ovule.

Suspensor haustoria which grow into the tissues of the ovule are also found in Rubiaceae, Fumariaceae, Crassulaceae, Tropaeolaceae and some other families.

The suspensor serves as a conduit for transfer of nutrients from the ovular tissues into the embryo. In *Capsella bursa-pastoris* and *Diplotaxis erucoides* for instance, the embryo has a uniseriate suspensor in which the micropylar cell is large and haustorial. The wall of this cell shows finger like wall ingrowths which expand the surface area of plasmalemma to promote greater intake of nutrients (Fig. 5.13). The cell walls separating individual suspensor cells are transversed by plasmodesmata. Cytoplasm of the suspensor cells has well developed endoplasmic reticulum and a large number of ribosomes, dictyosomes, mitochondria and plastids. Often the suspensor cells also show a high degree of endopolyploidy or polyteny. You will recall that these are characteristic features of transfer cells which are involved in short distance transport of metabolites. Hence, the suspensor participates in absorption nutrients and providing them to the morphogenetically more important part of the embryo. When the developing pods of *Phaseolus coccineus* are supplied with C-14 labelled sucrose, then the radioactivity is observed first in the suspensor and later in the embryo proper (Yeung, 1980). This demonstrates that the suspensor is the site of uptake of nutrients in the young embryo. At later stages the cotyledons themselves absorb nutrients from the endosperm. The suspensor thus acts as a temporary 'embryonic root' for nutrition of the embryo.

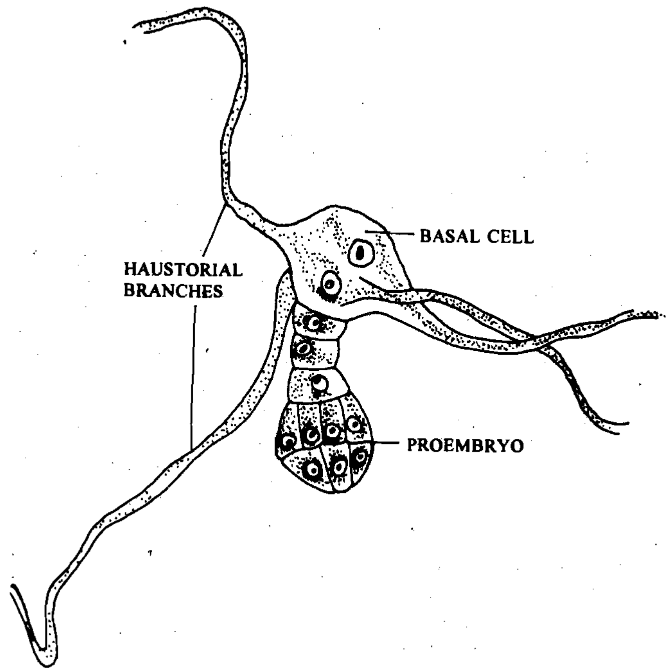

Fig. 5.12: Globular proembyo of *Dicraea*: The basal cell enlarges and gives out haustorial branches (after Mukkada, 1962).

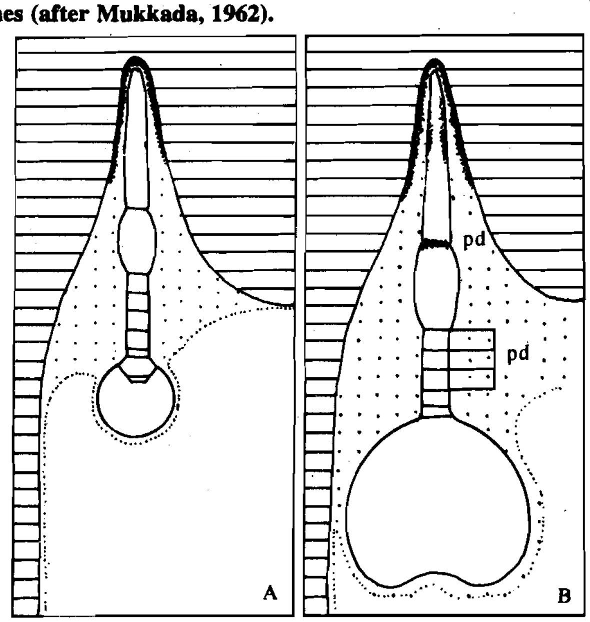

Fig. 5.13 A, B: *Diplotaxis erucoides*, diagrammatic representation of globular proembryo (A) and heartshaped embryo (B). Plasmodesmata (pd) occur on the transverse walls separating individual suspensor cells. (After Simoncioli 1974).

The suspensor starts degenerating from the heart-shaped stage of the embryo onwards. This is manifested by the disorganisation of cytoplasm and rupture of vacuolar membranes.

The suspensor is also considered as an important source of growth regulators. Suspensor cells have significantly amounts of plant growth regulators (PGRs) such as gibberellins, auxins and cytokinins. PGRs are believed to be supplied at specific stages to the embryo proper for regulating development. It has been observed in *Phaseolus* and *Eruca* (Corsi, 1972; Yeung & Sussex, 1979) that when a young embryo is cultured in an artificial medium without its suspensor, growth is retarded. However, at later stages of embryo removal of suspensor has no effect on the growth of cultured embryos. After the formation of cotyledons the level of gibberellin in the suspensor is reduced dramatically and there is a significant rise in its level in the embryo proper. This indicates that gibberellin has been transferred from the degenerating suspensor cells to the embryo proper. Nagl (1973) has aptly compared the embryonal suspensor in plants to the mammalian trophoblast which acts as a supply line for the nutrition of the foetus.

SAQ 3

Given below are some statements regarding the formation and function of suspensor. Put a (√) mark against those statements which are correct and cross (X) those which are wrong in the boxes provided:

a) The basal cell of the 2-celled proembryo generally contributes to the formation of the suspensor. [ ]

b) Growth of the suspensor is generally maximum after heart shaped stage of development of embryo. [ ]

c) In developing seeds of members of Fabaceae and Orchidaceae, a well developed suspensor helps in pushing the embryo deeper into the nutritive endosperm. [ ]

d) Suspensor cells often display a high degree of polyploidy. [ ]

e) Suspensor is rich in hormones that influence the growth and differentiation of embryo. [ ]

5.9 NUTRITION OF EMBRYO

Now you will study ***in vivo* and *in vitro*** studies in nutrition of embryo.

5.9.1. *In Vivo* Studies

The young proembryo derives its nutrition from ovular tissues with the help of suspensor. As the embryo develops its suspensor degenerates. Later the chief source of nutrition of the embryo inside the developing seed is the endosperm. By the time the proembryo attains a late globular state, and its suspensor becomes disfunctional, the endosperm is generally already a cellular tissue surrounding the embryo on all sides. The developing seed is a powerful sink for nutrients. Food materials received through the funicular vascular supply, are absorbed by the endosperm and passed on to the embryo for its growth and development. The central cell wall has transfer cells for absorption of nutrients. Sometimes persistent antipodal cells or special structures such as hypostase and postament of the ovule (about which you have studied in unit 2) help in translocation of food to the endosperm. After the embryo is fully grown, the endosperm persists in a large number of plants, particularly monocotyledons, storing starch or oil or protein or all the three. These are used up during seed germination. In other plants no endosperm may persist in the mature seed as the cotyledons store food reserves. Legumes are the best examples and have been the subject matter of research on storage proteins in recent times.

5.9.2 *In Vitro* Studies

Studies involving culture of embryos excised of various stages of development (i.e., early globular proembryo, late globular proembryo or heart shaped and torpedo shaped embryos) in media containing various combinations/concentrations of nutrients and growth regulators have been very helpful in understanding the growth requirements of embryos.

Experiments using embryos of *Datura, Capsella* and a few of other plants have shown that the mature embryo can develop into a normal seedling when cultured in a nutrient medium. Torpedo-shaped embryos require salts of essential minerals dextrose and certain amino acids, vitamins and PGRs for successful growth. However, late globular or heart-shaped embryos can be cultured only on addition of coconut water (liquid endosperm of coconut) to the above medium. So far it has not been possible to isolate and grow the zygote or a few celled proembryo on an artificial medium. Young proembryos when cultured become formless and do not acquire the typical structure of a mature embryo. Raghavan (1966) recognised two phases of embryo development based on nutritional requirements.

i) Heterotrophic phase: During this phase, which may last up to the globular stage, the proembryo is dependent upon the endosperm (or the ovular tissues).

ii) Autotrophic phase: This may begin at the late heart-shaped stage when the embryo becomes fairly independent for its nutrition. As a result, isolated older embryos require only a simple nutrient medium (containing sucrose and minerals) to develop into an organised embryo.

5.10 POLYEMBRYONY

Presence of more than one embryo in a seed is termed polyembryony. The phenomenon, first discovered in orange seeds by Leeuwenhoek (1719), attracted considerable attention because of its potential for application in and horticulture. Polyembryony is broadly classified into simple and multiple, depending on whether the supernumerary embryos arise in one or more embryo sacs in the ovule. Simple polyembryony may be sexual or asexual. In sexual polyembryony embryos may originate from the fertilized egg and a synergid, or by budding or cleavage of the proembryonal cells or suspensor of the zygotic embryo. Asexual embryos are produced within the embryo sac without fertilization. Embryos may also originate from diploid nucellar or integementary cells of the ovule and grow into the embryo sac. These embryos are termed adventive or sporophytic. Multiple polyembryony involves production of accessory embryos from two or more embryo sacs in the same ovule. For example, in the sea Island cotton, *Gossypium barbadense*, fertilization of egg in one embryo sac stimulates the induction of embryo from an unfertilized egg in the adjacent embryo sac within the same ovule. Thus, diploid-haploid twin embryos are produced in the seed.

Some of the more common methods of formation of additional embryos in the seed are discussed below:

5.10.1 Embryos from Synergids

The synergids, which usually degenerate prior to or soon after double fertilization, are reported to give rise to embryos in *Argemone mexicana, Tamarix ericoids, Dioscorea composita* (Fig.5.14).

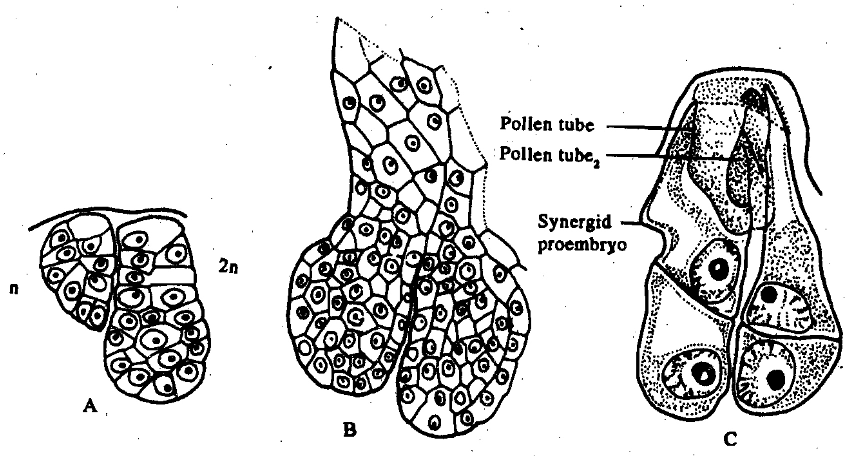

Fig. 5.14: Synergid polyembryony: A : *Argemone mexicana* B : *Tamarix ericoids* C : *Dioscorea composita.*

The synergid embryos grow along with the zygotic embryo in the embryo sac. In *Najas major* the synergid and the egg cell are fertilized and the resulting embryos resemble each other. Fertilization of the synergid is usually on account of entry of additional pollen tube in the embryo sac. Sometimes the unfertilized synergid is also stimulated to divide and form an embryo like structure. In the normal course only the zygotic embryo attains maturity and the haploid or diploid synergid embryo degenerates.

5.10.2 Zygotic or Suspensor Polyembryony

Cleavage of the apical cells of the globular or filamentous proembryo produced by the zygote may result in two or more embryos in a seed. Such (examples *Cocos nucifera* and *Primula auriculata*). This mode of polyembryony is also common among the orchids. Fig. 5.15A shows a group of cells produced from the zygote in *Eulophia epidendraea* which have developed into three distinct proembryos. In Fig. 5.15B an embryo is seen budding from a globular proembryo. In *Zygophyllum fabago* (Fig. 5.15C) and in several members of the Acanthaceae buds or new embryos arise from the uniseriate suspensor of the young proembryo.

The multiple embryos that arise from proembryonal or suspensor cells are diploid.

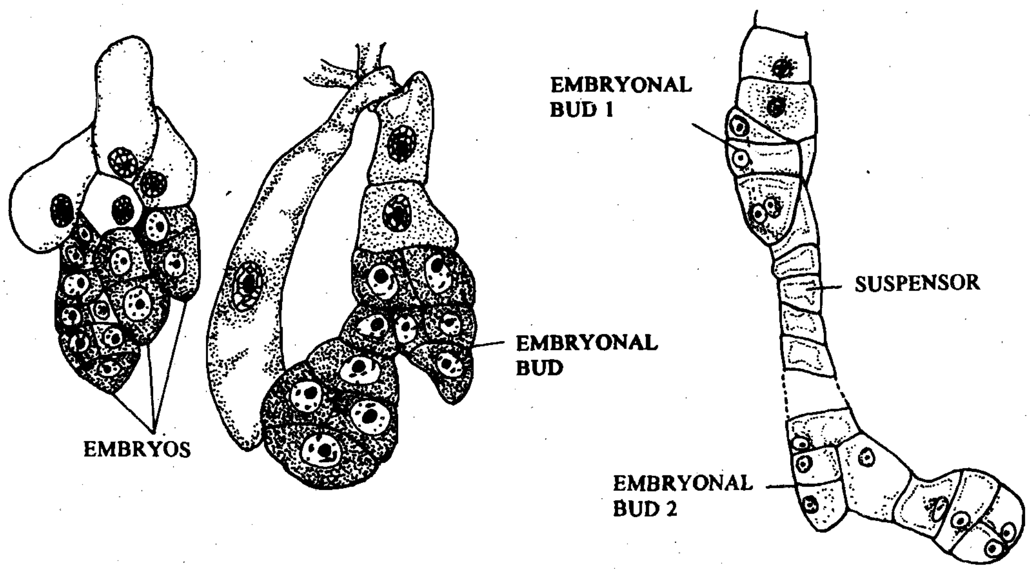

Fig. 5.15: Zygotic or Suspensor polyembryony. A. Zygote has produced a group of cells three of which have divided to form independent embryos. B. Budding of an embryo from a globular proembryo C. Proembryo with embryonal bud arising from uniseriate suspensor.

5.10.3 Nucellar Polyembryony

Members of families, Rutaceae, Anacardiaceae, Cactaceae, Myrtaceae and Orchidaceae, have a marked tendency for nucellar polyembryony. For example, in *Citrus microcarpa*

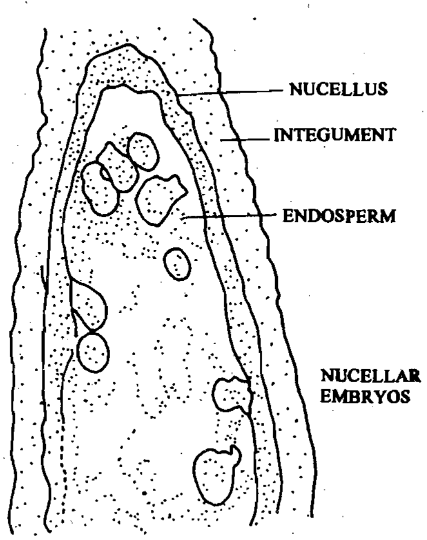

Fig. 5.16: Nucellar polyembryony.

in certain nucellar cells in the micropylar region become conspicuous by their denser cytoplasm and large nuclei. These cells divide repeatedly to form embryonal structures which project into the embryo sac. There can be 9-21 nucellar proembryos in an embryo sac (Fig. 5.16), which has a normal zygotic proembryo and endosperm resulting from double fertilization. The nucellar or adventive embryos pass through globular, heart-shaped and torpedo-shaped stages before undergoing complete differentiation as dicotyledonous embryos.

In the various species of *Citrus,* nucellar embryos are of great horticultural importance as the plantlets they produce are more vigorous, virus-free and endowed with well-developed tap root system in comparison with the shoot cuttings of the mother plants. Moreover, the seedlings are more uniform than those obtained through seeds.

In mango, *Mangifera indica* polyembryonate seeds may contain as many as 50 embryos. The embryos originate from nucellar cells in the micropylar region and grow into the embryo sac (Fig.5.17). When zygotic embryo is also present, it is difficult to distinguish it morphologically from the nucellar embryos. The varieties which form adventive embryos can be propagated by seeds which give seedlings that are of the same quality as the parent stock.

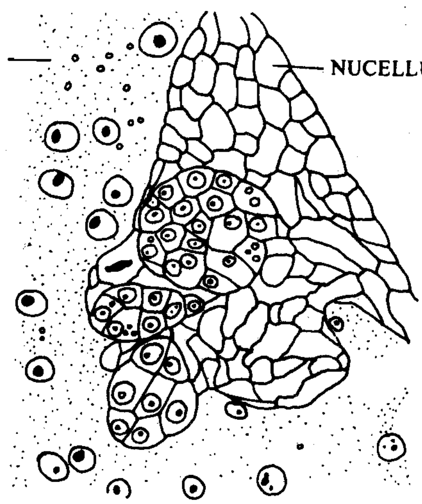

Fig. 5.17: Adventive proembryos.

5.11 USES OF PLURAL EMBRYOS

Although the basic that trigger causes polyembryony are not fully understood, there has been no dearth of interest in exploiting supernumerary embryos. The multiple embryos within a seed can be haploid, diploid or triploid. Haploid seedlings can be utilized for obtaining homozygous diploid forms by doubling the chromosome complement by a polyploidising agent. Such haploids and double haploids or homozygous diploids are of immense use in breeding superior crop varieties and hybrids. Adventive embryos are useful in agriculture and horticulture because they are genetically uniform and generally disease free.

In view of the utility of polyembryony, there have been several attempts to induce it artificially in those plants which are not normally polyembryonate or where the phenomenon occurs only occasionally. Environmental factors are often responsible for induction of polyembryony. However, application of PGRs has not proved successful in inducing polyembryony.

During the last three decades tissue in culture technology, involving excision of a desired tissue or organ and growing it on a nutrient medium under sterilized conditions, has been highly successful. It has helped in large scale propagation of plantlets obtained from vegetative or reproductive parts of several plants of agricultural and horticultural importance. Plantlets can now be obtained from the diploid tissues of nucellus or embryo or even from haploid micropores and triploid endosperm. Because of the wider application of tissue culture methodology, early interest in induction of polyembryony in field grown plants has died down.

SAQ 4

Fill in the blanks in order to convey a correct meaning of each sentence.

a) Formation of more than one embryo in a seed is termed ....

b) Asexual embryos arise without the intervention of ....

c) In *Argemone mexicana* additional embryo may arise from ....

d) Cleavage polyembryony is characteristic of the family ....

e) Nucellar cells destined to produce embryos have denser cytoplasm and .... nuclei.

f) Plantlets from adventive embryos are superior to vegetative cuttings in having .... root system.

5.12 SUMMARY

- You have studied in this unit that the zygote, gives rise to the embryo. The zygote undergoes a period of rest during which it shrinks, develops a complete wall around it and becomes polarized. Prior to division, it has a high rate of metabolic activity.
- With rare exception the zygote invariably divides transversely. Derivatives of the basal cell generally gives rise to the suspensor and those of the apical cell contribute to formation of different parts of the mature embryo. The four-celled proembryo is linear or T-shaped.
- Based on the plane of the first two divisions in the zygote, and the relative contribution of the daughter cells to different parts of the embryos, six embryogenic types are recognised — Piperad, Onagrad, Asterad, Solanad, Caryophyllad and Chenopodiad.
- Tangential divisions in the cells of the octant bring about histogenic stratification into three distinct layers — dermatogen, periblem and plerome.
- Precursors of various organs — cotyledons. The first change in the globular embryo is the initiation of cotyledons subsequent to which hypocotyl, stem apex and root tip — become evident in the organized embryo during maturity following globular stage of development.
- Proembryo receives nourishment through the suspensor. At later stages the embryo derives it from the endosperm.
- The suspensor undergoes various modifications in certain groups of plants, specially those which lack endosperm, to make it an effective source for absorption and transport of food materials for the embryo proper.
- A seed may have more than one embryo. Polyembryony may result from synergids or by cleavage of zygotic embryo. Adventive embryos also arise from the proliferation of nucellar or integementary cells. Nucellar embryos are of horticultural importance as they are true to the material parent and possess several describable features.

5.13 TERMINAL QUESTIONS

1) List the ultra structural changes that occur in the egg as a result of syngamy.
2) On what basis is embryogeny classified into six major types?
3) The 4-celled proembryo in *Capsella* has inverted T-shaped configuration. Describe the further course of development which leads to formation of a mature dicotyledons embryo.

4) Highlight the differences in development of a dicotyledonous embryo and a monocotyledonous embryo.

5) What are the parts of a fully organised dicotyledonous embryo? Trace their destiny.

6) Describe some features of the suspensor which make it suitable for absorpiton and transfer of nutrients to embryo.

7) Enumerate some modifications of the suspensor.

8) What is the role of endosperm in nourishment of the embryo?

9) What is polyembryony? Describe the important types of polyembryony in flowering plants.

10) Name the applications of polyembryony in horticulture

5.14 ANSWERS

SAQ 1

a) F, b) T, c) F, d) T, e) F.

SAQ 2

a) X, b) √, c) √, d) X, e) X.

SAQ 3

a) √, b) X, c) X, d) √, e) √.

SAQ 4

a) polyembryony
b) fertilisation
c) synergid
d) Orchidaceae
e) larger
f) tap.

Terminal Questions

1) In the fertilised egg or zygote a complete cell wall is formed. Cytoplasm becomes more polarized. There is an increase in the density cytoplasmic organelles. Refer to Section 5.2.

2) Six types of embryogeny are recognised on the basis of:

   i) plane of division of the zygote and of the cells of the 2-celled proembryo;

   ii) relative contribution of the derivatives of the 4-celled proembryo the formation of suspensor and parts of the mature embryo.

3) In the 4-celled proembryo of inverted T shape, each of the two terminal cells is divided by a vertical wall to form a quadrant. The quadrant cells in turn divide transversely to form an octant. Derivatives of the lower four cells of the octant give rise to the stem tip and cotyledons, whereas cells derived from the upper four cells of the octant form the hypocotyl. Derivatives of *ci* and some cells cut off from the intermediate cell *m* of the 4-celled proembryo divide to form a row of 6-10 suspensor cells. The lowest cell derived from *m* is referred to as hypophysis. It divides transversely to produce initials of the root cortex and of the root cap and epidermis.

4) Initial development of the proembryo is similar in dicotyledons and monocotyledons. However, during subsequent development in monocotyledons one half of the

half of the terminal cell and its derivatives have retarded growth. Derivatives of the other half grow rapidly to form one cotyledon. As a result, the stem tip which is also derived from the terminal cell appears to be lateral in position. According to Lakshmanan (1972) two adjacent cells of the terminal quadrant of the proembryo in Amaryllidaceae and Potomogetonaceae, three cells of the quadrant in Iridaceae and Potomogetonaceae, and all four cells except a few derivatives of one of them in Philydraceae contribute to formation of one cotyledon. The remaining portion or cells of the quadrant give rise to the stem tip.

5) A dicotyledonous embryo has an embryonal axis having two cotyledons, which expand into a pair of leaf like structures of the seedling. The portion of the embryonal axis above the level of cotyledons is termed the epicotyl which terminates in the plumule or stem tip. The cylindrical portion below is known as hypocotyl. It terminates at the other end in the radicle or root tip, covered by a well defined root cap.

6) The vesicular micropylar cell of the suspensor has transfer cell like structure. Cell walls separating individual suspensor cells have plasmodesmal connections. Cytoplasm of the suspensor cells has well-developed endoplasmic reticulum and a high density of ribosomes, mitochondria and dictyosomes. Suspensor, cell nuclei often show high levels of polyploidy. These characteristics make the suspensor highly suitable for absorption of nutrients from ovular tissues and transferring these to the embryo.

7) In *Pisum sativum* the suspensor consists of four large, multinucleate cells. In *Cytisus laburnum* cells of the suspensor are clusterd like a bunch of grapes. Such modified suspensors in Fabaceae are considered haustorial in nature.

Among the Orchidaceae the suspensor may be:

i) single-celled, vesicular (e.g., *Vanda*)

ii) branched, filamentous (e.g., *Ophrys*)

iii) like a bunch of grapes (e.g., *Epidendrum*)

iv) in the form of eight finger-like extensions enveloping the upper half of the embryo (e.g., *Vanda*).

v) Irregular mass of cells of which some micropylar cells elongate and form tubular extensions (e.g., *Cymbidium*) In number of the Rubiaceae, Fumariaceae, Crassulaceae and Tropaeolaceae also the suspensor cells form aggressive haustoria which grow into the tissues of the ovule.

8) Food materials received from the funicular vascular supply is absorbed by the endosperm from the ovular tissues and passed on to the embryo for its growth and development. In certain plants endosperm is fully used up during such development. In many others even after the embryo is fully grown, the endosperm stores large quantities of food materials in the form of starch or lipids or protein bodies or all of these. This reserve food is utilized by the embryo during germination of the seed.

9) Presence of more than one embryo in a seed is termed polyembryony. Polyembryony is simple if additional embryos arise in the same embryo sac, and multiple if they are formed in more than one embryo sac in the ovule. In sexual polyembryony embryos may originate from the fertilized egg and synergid (e.g., *Najas*), or by budding or cleavage of the suspensor (e.g., *Diptera-canthus*) or proembryonal cells (e.g., *Eulophia*) of the zygotic embryo. Asexual embryos are produced in the embryo sac without the process of fertilization. More commonly multiple embryos may develop from the diploid nucellar (e.g., *Citrus* and *Mangifera*) or integumentary cells of the ovule. Such embryos are termed adventive embryos.

10) Multiple embryos can be haploid or diploid depending on whether they originate from an unfertilized gameotophytic cell such as a synergid or an egg, or a sporophytic tissue such as nucellus or integument. The haploid, double haploid and homozygous diploid obtained from it, are of immense use in breeding of super crop varieties. Adventive embryos are useful in horticulture because they can generate uniform and disease free seedlings.