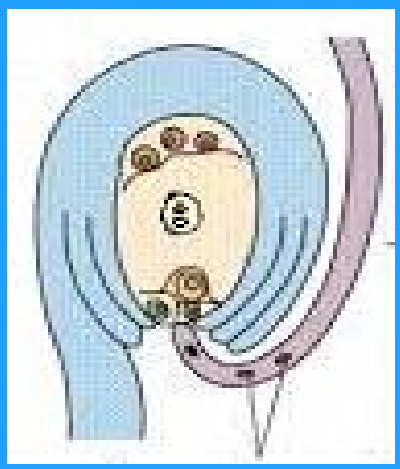
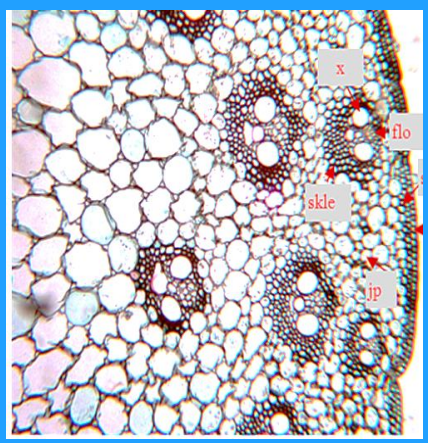
Basics of Plant Anatomy, Embryology and Tissue Culture





Dr. Azahar Sajjad
Dr. Akil Ahmad Khan

Basics of Plant Anatomy, Embryology and Tissue Culture

EMPYREAL PUBLISHING HOUSE
India | UAE | Nigeria | Uzbekistan | Montenegro

Basics of Plant Anatomy, Embryology and Tissue Culture

By:

Dr. Azahar Sajjad
Assistant Professor
G. F. College, Shahjahanpur

Dr. Akil Ahmad Khan
Assistant Professor
G. F. College, Shahjahanpur

First Impression: 2020

Basics of Plant Anatomy, Embryology and Tissue Culture

ISBN : 978-81-946373-7-0

Rs. 650/- ($18)

DISCLAIMER

Information contained in this book has been published by Empyreal Publishing House and has been obtained by the author from sources believed to be reliable and are correct to the best of her knowledge. The author is solely responsible for the contents of the articles compiled in this book. Responsibility of authenticity of the work or the concepts / views presented by the author through this book shall lie with the author and the publisher has no role or claim or any responsibility in this regards. Errors, if any, are purely unintentional and readers are requested to communicate such error to the author to avoid discrepancies in future.

Published by:
Empyreal Publishing House

Dedications

Dedicated to my father whose unending love and care even a day before his death still haunts me that only father's love, affection and supports are real.

Dr. Azahar Sajjad

Dedicated to my beloved parents, brother, sisters and teachers.

Dr. Akil Ahmad Khan

Preface

The book has been written for the comprehensive treatment of angiosperms containing anatomy, embryology and some basics of tissue culture which is divided into different relevant chapters for easy understanding of the concerned topics and covers the basic concept related to plant anatomy, embryology and tissue culture.

This book is intended to fulfill the need of students of graduate and up to some extent post graduate level of all Indian Universities. All the three texts mentioned above have been discussed sufficiently. The text has been written in simple, lucid and graspable language and also illustrated with self explanatory diagrams.

The purpose of this book is not only to solve the problems of students in universities examinations but also useful for various competitive examinations. The book has been written with proper care; however, the authors will be very grateful for any suggestions and comments for the improvement of this book.

Acknowledgements

We are grateful to Honorable Syed Moinuddin Mian, President, Management Committee, G. F. College, Shahjahanpur for his love and affection towards the college staff. We are also thankful to prof. Nasimusshan Khan, Principal of the college for his continuous encouragement. It is our immense pleasure to acknowledge the support and encouragement of our colleagues; Dr. S. S. A. Naqvi, Dr. Mohammad Sayeed Akhtar and Ms. Shazia Bi. We are also grateful to Dr. Areeb Anjum Rehman, Dr. Abul Hasnat, Dr. Musharraf Ali, Dr. Arshad Ali, Dr. Jamil Ahmad Khan, Dr. Mansoor Ahmad Siddiqui and others honorable members of the college for their best wishes and suggestion from time to time. The authors are also thankful to Prof. Samiullah (former professor and chairman, Department of Botany, A.M.U.; Aligarh) for his continuous motivation. Above all the authors will always be indebted to Prof. Nafees Ahmad Khan (Professor and chairman, Department of Botany, A.M.U.; Aligarh) for his endless support, love and faith. We also acknowledge to our friends Dr. Ajay Prakash, Dr. Hitendra Kumar (G. R. College, Rampur) and Dr. Adarsh Pandey (Head, Dept. of Botany, S.S. College, Shahjahanpur) for their valuable suggestion during the preparation of this book.

We also acknowledge to Mr. Syed Anees Ahmad, librarian of the college for providing necessary literature and also to Mr. Samad Khan, office superintendent of the college for his valuable support.

We are also thankful to non-teaching members of our departments for their love and support.

Last but not the least we also pay our thanks and gratitude to the friends and loving family members for their best and sincere wishes and co-operation.

Dr. Azahar Sajjad

Dr. Akil A. Khan

Table of Contents

Chapter-1

Introduction and Meristematic tissues

Introduction

Plant anatomy is the branch of science which deals with the study of gross internal structure of plant organs as observed after section cutting. It explains about the different types of tissues and tissue systems in the plant body. Histology means study of tissues is also a part of anatomy. **Theophrastus** was the first person who recognized bark, medulla and xylem (Zylon). The anatomy came into light after the invention of microscope. Study of this branch started in 1671, when **Marcello Malpighi** and **Nehemiah Grew** independently studied the anatomy of vegetable plants and laid the foundation to anatomy. Grew (1671) introduced the terms like parenchyma, cortex and vessels in his book, **'Anatomy of Vegetable'**. Malpighi (1675) coined the terms like epidermis, stomata in his book, **'Anatome Plantarum'.** Nehemiah Grew is known as Father of Plant Anatomy. K. A. Chaudhary is known as Father of Indian Plant Anatomy.

Plant Tissue

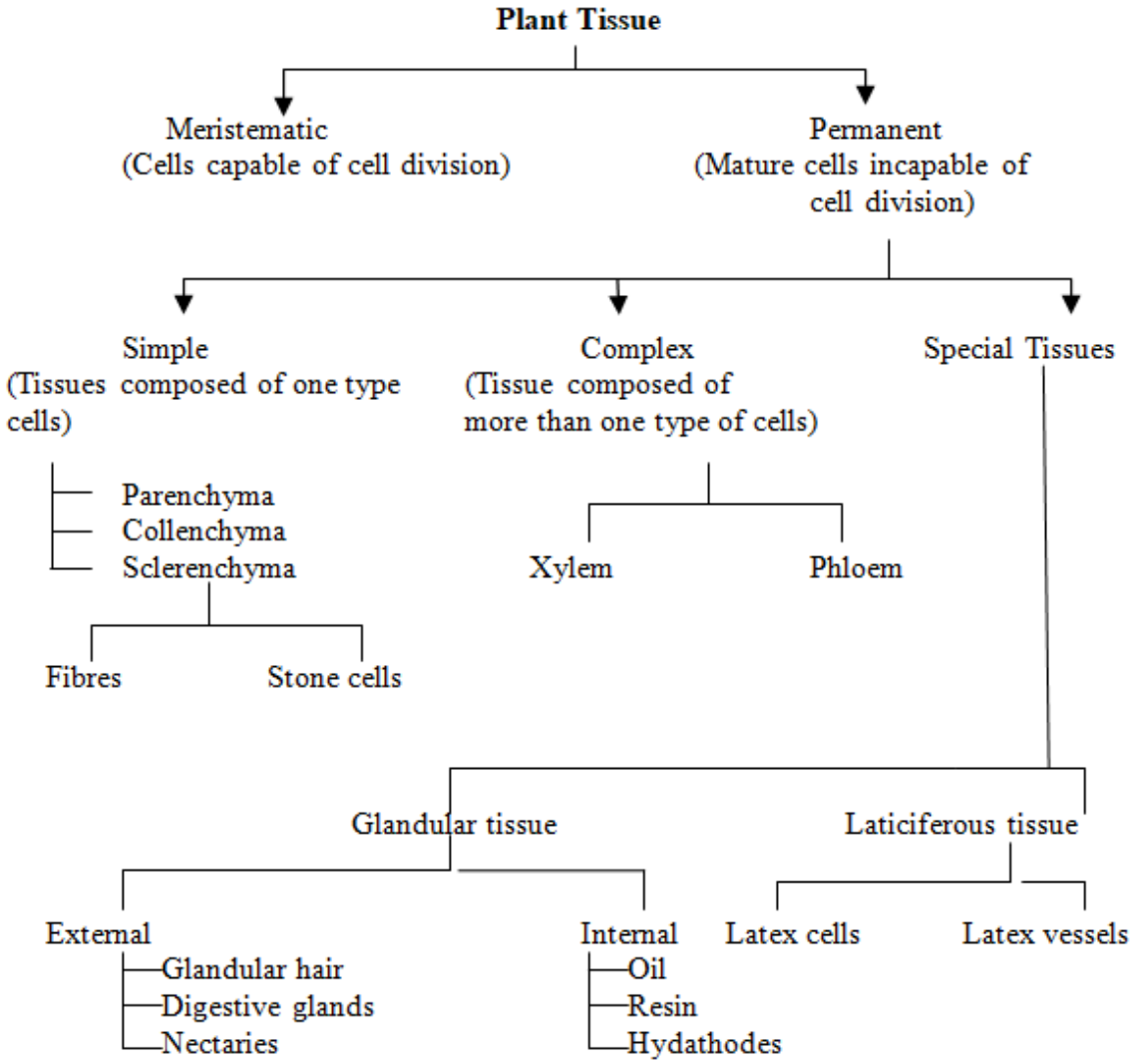


Fig. 1.1, Flow chart showing tissues and their types

Tissues: Tissue is a group of similar or dissimilar cells of common origin that perform or help to perform a common function. A tissue is really formed in response to a basic division of labor. The cells constituting a tissue are connected together by plasmodesmata for proper co-ordination amongst

them. The term **tissue** was coined by **Nehemiah Grew.** In Plants, anatomy is considered equivalent to histology because in plants not much internal structure is visible to naked eyes. The term **histology** (study of tissues) was introduced by **Mayer**.

Types of Tissues: Based on the capacity to divide **Carl Van Nageli** (1858) classified plant tissues into two main groups: Meristematic tissues (undifferentiated tissues) and Permanent tissues (fully differentiated tissues).

Meristematic Tissue (Meristems): The term meristem has been derived from the Greek word meristos – which means divisible or having cell division activity, so, meristem is a group of cells that are in a continuous state of division or retain their power of division e.g. meristem at the apex of root, stem, vascular cambium etc.

Characteristics of Meristems

1. The cells having ability to divide and re-divide again & again constitute the meristematic tissue.

2. Meristematic cells have only primary cell wall (made of cellulose) which is thin and flexible and lack secondary and tertiary cell wall.

3. Meristematic cells are of various shapes like oval, spherical, rounded or rectangular.

4. Cells are small having dense cytoplasm, vacuoles are small & many or generally absent, however, the cells of cambium are vacuolated, large with prominent nucleus, metabolically highly active, and so is the rate of respiration.

5. No reserve food and no intercellular spaces are found. Cytoplasm has all the cell organelles but plastids absent (if present, only at pro-plastidial stage), mitochondria & ER are very much simple in structure (internal structure not developed) and ergastic substances are also absent. Ribosomes are abundant.

6. Meristematic cells of vascular cambium are elongated and contain ergastic substances like starch grains and tannins.

Classification of Meristematic Tissues: Generally the meristematic tissues can be classified on the basis of:

A. Origin & development

B. Position in the plant body

C. Functions in the plant

D. Plane of division

E. Rate of division

A. Classification of meristems on the basis of origin and development: On the basis of origin and development, the following three types of meristems have been recognized:

1. **Pro-meristem:** They are also called primordial or Ur-meristem or embryonic meristem. They are present in small region at the apices of shoot & roots (tip of plumule and radicle) where the foundation of new organs or their parts is laid down (Found in the embryo of seed). It gives rise to primary meristem.

2. **Primary meristem (Eu-meristem):** Primary meristem originated from pro-meristem (i.e. They are the direct descendents of embryonic cells) and found below it at shoot & root apices, apex of leaves, primordial of leaves, in intercalary parts and in intra-fasicular cambium in dicot stem and stem of gymnosperms. It is always in active state of division and divides in all possible planes producing new cells. Apical meristem, intercalary meristem and intra-fascicular cambium are primary meristem. A whole plant can be built by the activity of primary meristem and in most of the monocots and herbaceous dicots, it is the only type of growth.

3. **Secondary meristem:** The meristem which appears later during the stage of development of plant organs is called secondary meristems. This meristem developed from primary permanent tissue (mature tissues which have already undergone differentiation) when the permanent tissue become meristematic. They divide and form secondary permanent tissue e.g. Cambium of dicot root, cork cambium (phellogen) in stem and root, inter-fasicular cambium in stem, wound cambium etc. Secondary meristems are lateral in position and increase the girth of the plant.

B. Classification of meristems on the basis of position in the plant: On the basis of their position in the plant body, meristems have been classified into the following three groups:

1. **Apical Meristem**: It is present at the growing tips of stems and roots and increases the length of the stem and root. They form growing parts at the apices of roots and stems and are responsible for increase in length. This is also called **primary growth**. This meristem is responsible for the linear growth of an organ.

2. **Intercalary Meristem:** This meristem is located in between permanent tissues. This is the separated region from the apical meristem which is left behind during the growth. It is nothing but the part of apical meristem. It may be present at base of the internodes (e.g. *grasses, wheat, bamboo* etc), or the base of leaves e.g. *Pinus***,** or nodes e.g. *Mint* or *Mentha* (Labiatae). It is responsible for growth in length mainly internodal elongation. Intercalary meristems are short lived. On losing their power of division, they merge with permanent tissue. Grasses and Bamboo grow in length by the activity of this meristem.

3. **Lateral Meristem (Cambium):** It is present in lateral side of plant organ i.e. parallel to long axis of plant organ. Lateral meristem divides only periclinally (radially) and is responsible for growth in girth or diameter. It is responsible for secondary growth. Lateral meristems are both, primary and secondary in origin but mostly are secondary in origin. There are only two examples of Primary lateral meristem:

a) **Marginal Meristem:** It occurs at the margin of leaves and is responsible for the increase in the width of leaf.

b) **Intrafasicular Cambium:** Cambium present inside the vascular bundle.

Except this cambium, all cambium are secondary in origin e.g. cork cambium, cambium of dicot roots, wound cambium.

C. Classification of meristem on the basis of functions in the plants: According to Haberlandt (1880), the primary meristems in the root and shoot apices have been divided in three categories on the basis of their functions:

1. **Protoderm:** It is the outer most layer of apical meristem which gives rise epidermis of stem and epiblema of roots.

2. **Procambium:** Meristem which develops into primary vascular tissue is called pro-cambium. The derivatives of pro-cambium are differentiated into phloem, xylem and cambium.

3. **Ground meristem:** Meristem which develops into ground or fundamental tissue is called ground meristem e.g. cortex, endodermis, pith etc. More appropriately, it can be said that it forms all the rest part of the plant body except epidermis and vascular tissues.

Note: All these meristems are primary in nature as they form primary permanent tissues.

D. Classification of meristem on the basis of plane of cell division:

1. **Rib or File Meristem:** In this type cell division occurs at right angles or anticlinally in one plane. This results in the formation of long rows or files of cells. Such meristem play important role in the development of young roots as well as cortex and pith of young shoots.

2. **Plate Meristem:** It consists of parallel layers of cells which divide anticlinally in two planes so that a plate like structure is formed e.g. epiblema, epidermis, endodermis, pericycle. The leaves of the plants are formed through this type of meristem.
3. **Mass Meristem:** Meristem divides in all planes and forms a mass of cells and increase in volume of plant organ e.g. formation of embryo and endosperm etc.

E. Classification based on rate of division: According to **Foster,** meristem classified into two regions on the basis of rate of division. In vegetative shoot apex, there occur two zones:

1. **Summit:** In this region rate of division is slow. This region is located at the apex.
2. **Flanks:** In this region, rate of division is very fast. This is located behind summit and leaf primordial is formed by this region.

Plastochoran: Time period / gap between initiations of two successive leaf primordial is called **plastochoran.** During reproductive phase i.e. at the time of flower formation, vegetative shoot apex, transforms into reproductive shoot apex. In reproductive shoot apex, the summit zone is more active i.e. rate of division is more and it forms stamen and carpel and flanks zone is less active in reproductive shoot apex and it forms sepals and petals.

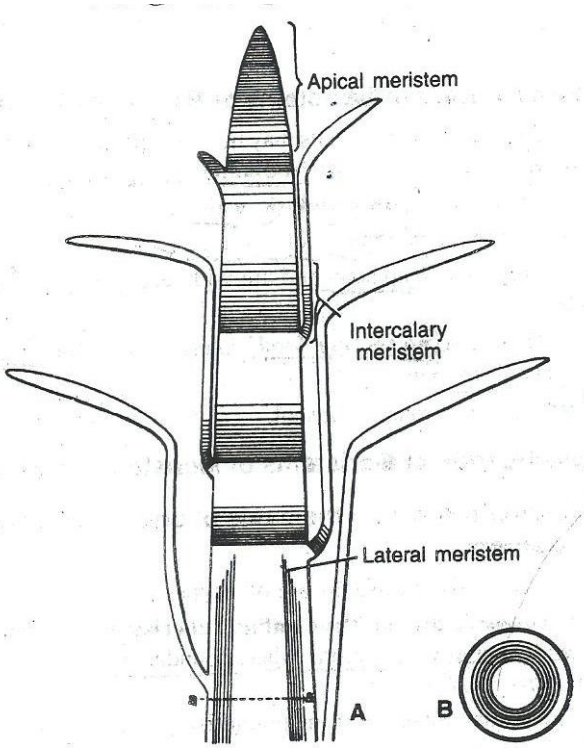


Fig. 1.2, Different types of meristems on the basis of position in plant body

A. L.S, B. T.S.

Table: 1.1, Difference between primary and secondary meristems

S. No.	Primary Meristem	Secondary Meristem
1.	This is present from the beginning.	This is formed later during the development plant.
2.	Dedifferentiation is not involved.	Permanent cells are transformed into meristematic cells through dedifferentiation.
3.	Cells are small and isodiametric. (Except intra-fascicular cambium).	Cells are elongated.
4.	Central vacuole is absent	Cells possess central vacuole.

5.	It is responsible for primary growth. (Except intra-fascicular cambium cells, the cells of primary meristem add growth in length).	It is responsible for secondary growth i.e. add growth in thickness (width).
6.	Develops from pro-meristem.	Develops from the mature (permanent) tissue due to dedifferentiation.

Table: 1.2, Difference between apical and lateral meristem

	Apical Meristem	**Lateral Meristem**
1.	Present at apex of root, shoot and their branches.	present in lateral position parallel to circumference
2.	It is primary meristem.	It is secondary meristem except intrafasicular cambium
3.	Cells divide in different planes.	Cells divide in one plane i.e. periclinally both on outer and inner side
4.	Produces primary tissues.	Produces secondary tissues.
5.	Brings about growth in length.	It causes growth in thickness.

Apical Meristem: In vascular plants, the meristem which first appears in embryonic shoot or embryonic root is known as apical meristem. All primary tissues of the plant body originate from the shoot and root apical meristem. Apical meristem can be discussed under the heads – vegetative shoot apical meristem (vegetative shoot apices), reproductive shoot apical meristem (reproductive shoot apices) and root apical meristem. Apical meristem is without vascular tissue, remains virus free and can be used as explants in tissue culture to get virus free plants. Apical meristem presents at the tip of root & stem and is responsible for increase in length. Apical meristem is absent in lower plants i.e. algae and fungi. All the cells of these plants are divisible; such type of growth is called **diffused growth.** Diffused growth is also occurring in animals. Apical meristem in bryophytes and some pteridiophytes consists of single cell. This cell is known as **apical cell** and is of pyramid shape**.** Apical meristem in Ferns, Gymnosperm and Angiosperm consist of many cells.

Shoot Apex Organization: The shoot apex is the terminal part of the shoot, which is immediately above the youngest leaf primordium. It is present at plumular tip or at the end of leaf. It is covered by young leaves and is visible on removing them. According to Foster, Gifford and Clowes "shoot apex is a portion of shoot above the youngest primordium". It is conical or dome shaped and shows rhythmic change in size.

Theories of Shoot Apex Organization: Several theories have been proposed from time to time to explain the organization of shoot apex. Some important theories are discussed below:

A. **Apical cell theory:** It was proposed by **Nageli** (1857) and was supported by **Hoffmiester** (1858). Nageli studied the apical meristem of *Dryopteris* (a pteridophyte) and put forth an explanation that the single apical cell is responsible for the shoot formation and successive growth of the plants. It was considered that the plant body arises from a single cell and its derivatives. This theory may hold good for Cryptogams (higher algae, bryophytes, some pteridophytes); but it is certainly not applicable to the seed plants. Further investigations stated that, different parts of plant body arise independently.

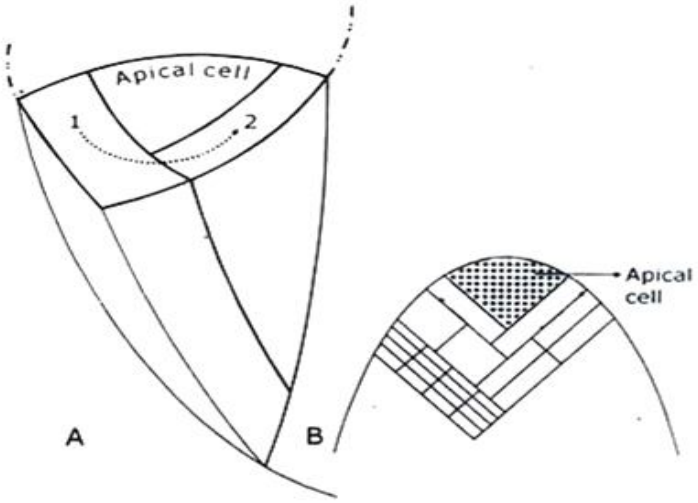


Fig. 1.3, A. Diagram showing an apical cell of leptosporangaite fern and its derivatives that are formed in helical succession. The new cells are numbered as 1 and 2. **B.** Diagram showing a packet of cells formed by an apical cell by division and subdivision.

B. **Histogen theory:** This theory was proposed by Hanstein (1870). He studied the apical meristem of onion and according to him, the root and shoot apices of plants consist of three meristematic zones called **histogen (Tissue builder)** which are as:

1. **Dermatogen:** It is the outer single layer which forms uniseriate epidermis by anticlinal division.
2. **Periblem:** It is the middle layer which forms hypodermis, cortex, and endodermis.
3. **Plerome:** It is the central core layer which forms the stele i.e. Pericycle, vascular bundle, medullary rays and pith.

Demerits: During further investigation, distinction into periblem and plerome in the apical parts of many angiosperms and gymnosperms has not been found. So it is not possible to assign to the histogens the origin of the various regions of plant body.

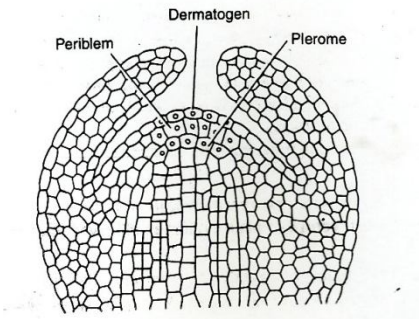


Fig. 1.4, Shoot apex organization according to histogen concept

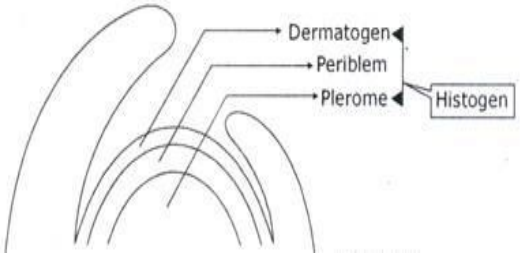


Fig. 1.5, Schematic diagram of shoot apex organization according to Histogen theory

C. **Tunica corpus theory:** It is the most accepted theory and was proposed by **Schmidt (1924).** This theory is applicable to shoot apex. Schmidt while working on the apical mersitem of *Vinca minor* recognized only two zones in the shoot apex of angiosperm which are as:

1. **Tunica:** This is the outer single layer and divides only anticlinally (only vertical division, surface area of plant organs increases due to anticlinal division, in this division only number of cells increases but not the layers). It is responsible for surface growth and produces epidermis, leaf and axillary bud primordial.

2. **Corpus:** The inner mass of the cells present below the tunica is called corpus. The cells divide in all direction (anticlinal & periclinal) resulting increase in volume and produces cortex, vascular tissues and pith. The corpus cells are larger than tunica. Generally tunica is single layered but some time it is multilayered. In case of multilayered tunica, the outer most layer forms epidermis and remaining layers form another type of tissue with association of corpus. According to this theory outer tunica gives rise to epidermis and inner tunica (if multilayered) and corpus give rise entire tissues including cortex and vascular tissues. Tunica and corpus can be distinguished on the basis of plane of cell divisions. The cells of tunica divide only anticlinally, while those of corpus divide in all planes. Corpus responsible for volume growth.

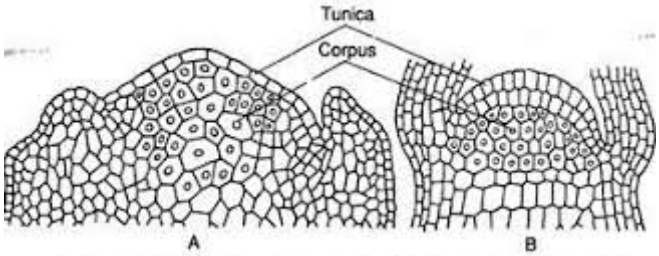


Fig. 1.6, L.S. through shoot apex showing tunica (A) and corpus (B)

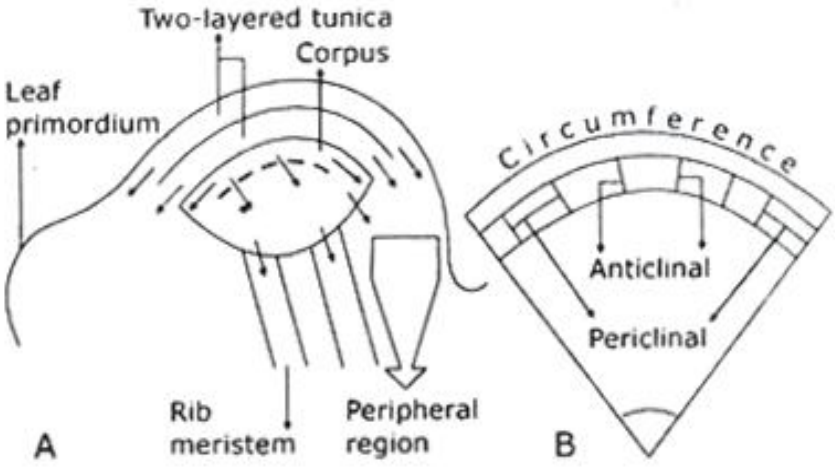


Fig. 1.7, A. Diagram illustrating the tunica-corpus organization of dicotyledonous shoot apex. Arrows indicate the direction of cell formation in apical meristem. **B.** Schematic representation of anticlinal (=division wall perpendicular to the surface) and periclinal (= division wall parallel to the surface) division.

D. **Mantle-Core Concept**: It was proposed by **Popham** and **Chan** (1950). This concept was formulated to support the tunica corpus theory. They used the term mantle for the outer dome shaped layers that covered the central part called the core. This theory is just substitute for tunica and corpus theory. Tunica is regarded as mantle and corpus as core.

1. **Mantle** (Tunica): It forms epidermis.

2. **Core** (Corpus): It is divisible into three regions which are as:

a) **Sub apical meristem:** It is present below mantle and help in re- establishment of mantle, when mantle damaged

b) **Central zone meristem:** It is responsible for the formation of pith.

c) **Peripheral meristem:** It is responsible for the formation of cortex and vascular tissues.

E. **Concept of meristen d' attente.** This concept was proposed by **Buvat**. He recognized only three regions in the apical meristem of the shoot. These regions were named as:

1. **Anneau initial or peripheral active zone**: It is the active region of shoot apex and gives tissues in the outer or peripheral regions of the stem e.g. epidermis, cortex and endodermis.

2. **Meristem d' attente or the waiting meristem:** It is active only during the formation of inflorescence or terminal flowers.

3. **Meristem Medullaire or the pith meristem:** It gives the vascular region and pith.

F. **Concept of Newman**: This concept was given by Newman (1961) who recognized three types of shoot apices:

1. **Monoplex**: It is found in ferns and its allies. The shoot apex may have one or more cells or initials.

2. **Simplex:** It is found in Gymnosperms. One or more initial cells arranged in single layer of the shoot apex. These cells divide both anticlinal and periclinal.

3. **Duplex**: It is found in Angiosperms. The shoot apex consists of two layers of initials. Outer layer divides in anticlinal plane and the inner is both anticlinal and periclinal planes.

Root Apical Meristem (Root Apex): The root apical meristem is relatively simple than shoot apex. It is sub-terminal in position because root cap (calyptrogens) is present at the tip. It is not associated with the formation of lateral appendages and differentiation of nodes and internodes. The root apical meristem (RAM) is a complex structure. *Arabidopsis thaliana* root is one of the ideal systems for the study of plant organogenesis. The primary root grows mainly by cell division. Cell elongation is also contributes to the root growth.

Theories of Root Apex Organization: Different theories of root apex organization have been put forth from time to time. The top three theories of root apical meristem in plants are: 1. Apical Cell Theory 2. Histogen Theory 3. Korper-Kappe Theory.

1.**Apical cell theory**: It was proposed by Nageli and supported by Hofmeister (1878). According Nageli a single tetrahedral apical cell in the root apices brings about growth and works as the structural and functional unit of the root apical meristem. The three upper sides form the root whereas the lower side forms the root tip. This theory is not applicable because this type of development is absent in Angiosperms.

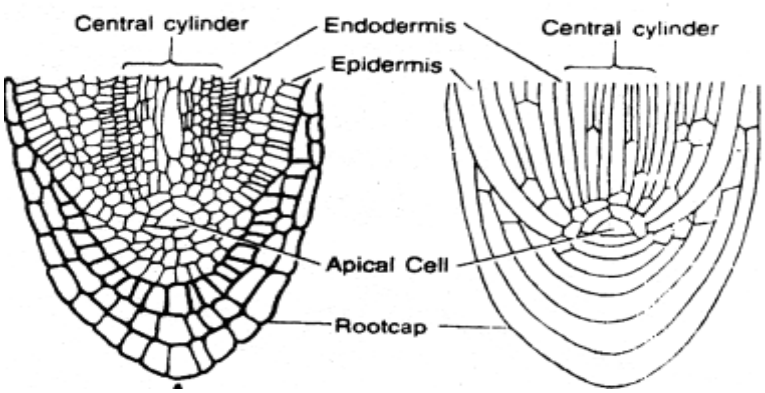


Fig. 1.8, Root tips of ferns showing apical cells

2. **Histogen theory:** This theory was proposed by **Hanstein** (1868). The three histogens are same as in case of shoot apex theory. In case of root apex, hanstein proposed one more histogen i.e. **calyptrogen** which form the root cap. The root apex is enclosed by root cap. Thus the apical meristem in roots is not terminal but it is sub-terminal. (Apical growth in case of root is sub-terminal and in case of stem is terminal). In monocot, a fourth layer **calyptrogen** gives rise to **root cap.** Root cap only produced by dermatogen in dicot.

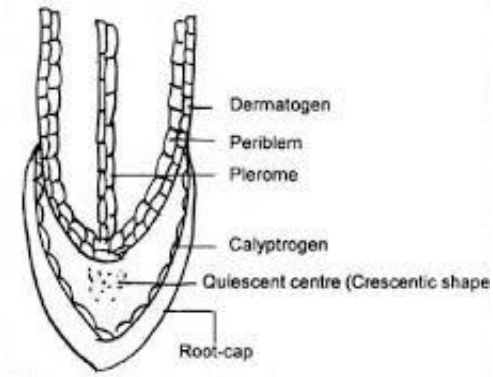


Fig. 1.9, Histogen theory – Schematic representation of organization of root apex

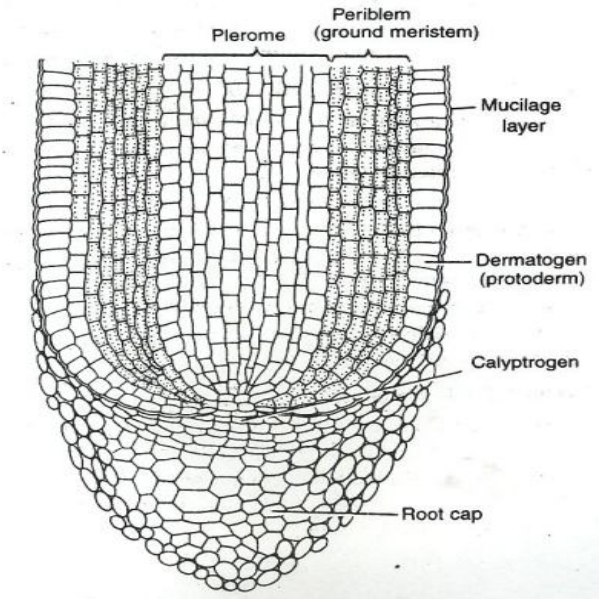


Fig. 1.10, Cellular representation of root apex organization according to Histogen theory

3. **Korper–Kappe theory:** This theory was proposed by **Scheupp (1917)** and is applicable to root apex only. It is almost similar to tunica-corpus theory of shoot apex. It is based on the differences of planes of cell division. According to him two zones, Korper and Kappe, can be distinguished in the root apex. Both these zones are characterized by peculiar type of cell division. The cells of root apex divide first by transverse wall and then one of the cell divide by vertical walls. Some cells form inverted T which is known as Kappe and represent the root cap while some other cells form straight T which is known as Korper and represent the body of the root apical meristem (central region). Korper-kappe concept is also referred to as body-cap concept (Korper = body and kappe = cap) and the concept illustrates distinct type of cell wall pattern formation during cell division. The body-cap concept is illustrated below on analyzing the divisions in the derivatives of apical cell (Fig.)

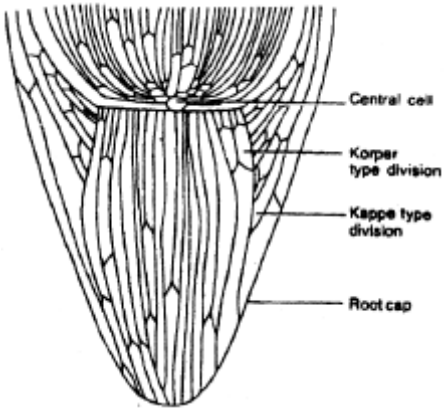


Fig. 1.11, Organization of root tip according to Korper-Kappe concept

4. **Quiescent centre concept:** This concept was proposed by **Clowes (1959-61)** who on the basis of autoradiographic studies, observed a cup like structure of inactive cells present in between dermatogen and calyptrogens in the root tip of *Zea mays* which is called **quiescent centre**. Cells of these regions normally remain inactive and act as reservoir of meristematic zone where divisions are very few as DNA synthesis is very rare. When meristem get injured (or calyptrogens get damaged), then it becomes active to compensate the losses and again become inactive. Quiescent centre characterized by having low DNA, RNA, fewer mitochondria, very small dictysomes, nuclei and nucleoli. Quiescent centre may contain 500 cells in *maize* and 1100 cells in *Vicia faba.*

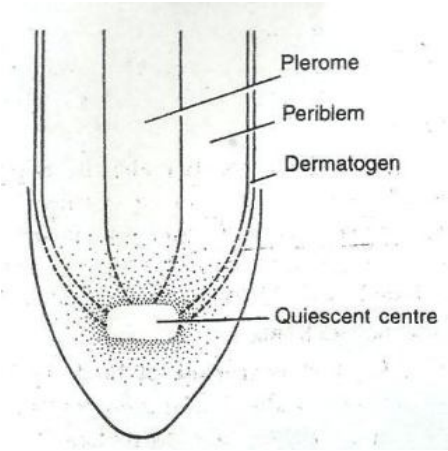


Fig. 1.12, Root apex organization according to quiescent centre concept

Chapter-2

Permanent Tissues

Permanent tissues: The tissue whose cells has lost their divisional capacity and has assumed a definite shape and size in response to varied mechanical and physiological function is called **permanent tissues.** Permanent tissues are formed due to division and differentiation in meristematic tissues. These cells may be living or dead; thin walled or thick walled. Thin walled cells are generally living where as the thick walled cells may be living or dead. The permanent tissues which developed from apical meristem and lateral meristem are called **primary** and **secondary permanent tissue** respectively. On the basis of constituent cell, the permanent tissues are classified into three types:

A. Simple permanent tissues

B. Complex tissues

C. Special tissues

A. Simple permanent tissues: Simple permanent tissue consists of cells which are homogenous in nature, having similar origin, structure and function. On the basis of the structure of constituent cells, simple tissues are of three types:

a) Parenchyma

b) Collenchyma

c) Sclerenchyma

a) **Parenchyma:** The term parenchyma was coined by Grew (1682). It is the most common, abundant, diverse, and versatile cells in a plant. It is the most primitive tissue from phylogenetic point of view and considered to be evolved first. It is considered the precursor of all tissues and hence also known as fundamental tissue. Parenchyma consists of usually isodiametric cells (equal diameters in all directions), polygonal cells, but the cells may be oval, rounded, and rectangular, cylindrical, star shaped or long spindle like and shape and size vary greatly in different plants or in different organs of the same plant. It is universal tissue, it means it is found in all parts of the plants e.g. leaf, stem, fruits, flower etc. Number of facets in polygonal cells may be as many as 10 - 14 along which they come in contact with neighboring cells. Pressure of neighboring cells and surface tension play significant role in determining their shape with in tissue. This characteristic is due to more flexibility. Cells are loosely arranged with many intercellular spaces (Exceptions are epidermis, epiblema, endodermis, pericycle and pith rays where cells are compactly arranged without any intercellular spaces). Intercellular spaces develop either schizogenously (by splitting apart of the middle lamellae region between cells) or less frequently lysogenously (by breakdown or lysis of cells). Primary and secondary wall, both are composed of cellulose, hemicellulose & pectin and are thin but the parenchyma cells of secondary xylem are thick due to the presence of lignin in secondary wall. Epidermal cells are cutinized and that of endodermal cells are suberized. Parenchyma cells of epidermis having cutinized cell walls are protective in function and prevent evaporation of water. The parenchyma cells show a thin layer of dense cytoplasm, a peripheral located nucleus and a large central vacuole. All meristems made up of parenchyma.

Modifications of parenchyma or specialized parenchyma: The modification of parenchyma is for different functions in plants. The modifications are as follows:

1. **Prosenchyma:** These are long parenchyma cells with both the ends tapered e.g. Pericycle (commonly) but can occur any where in plant. It provides strength and rigidity.

2. **Aerenchyma:** In hydrophytes, the parenchyma develop air spaces and such parenchyma with air cavities is known as aerenchyma e.g. *Eichornia, Hydrilla etc.* It helps hydrophytes to float and provide oxygen for respiration.

3. **Chlorenchyma:** Parenchyma containing chloroplasts and carry out photosynthesis e.g. mesophyll cells of leaf. Mesophyll cells consist of palisade parenchyma and spongy parenchyma. It is also known as assimilatory parenchyma.
4. **Stellate parenchyma (star like parenchyma**): It is found in the leaf base of banana and *Canna*. It provides strength to leaf base. Leaf base of banana performs the function of stem. Rhizome is found in banana.
5. **Mucilage parenchyma:** In the mucilage parenchyma, large vacuole and mucilage will be found e.g. xerophytic plants. Its major role is storage of water e.g. *Opuntia, Euphorbia, Aloe, Agave* etc.
6. **Transfer Cells**: Rapid transport of food metabolites associated with veins of leaves and nectaries of flowers.
7. **Idioblast cells:** Some cells of parenchyma store ergastic/waste material and called as idioblast cells. Idioblast cells contain oil, tannin, resin, alkaloids, gums, terpenes, crystal of Calcium oxalate in the form of food e.g. *Citrus, Eucalyptus* etc.

Origin of parenchyma: Parenchyma originates from the cells of meristem by losing their divisional capacity. Parenchyma is found in cortex, pith, mesophyll and some organs of flower are derived from ground tissues. Parenchyma present in vascular tissue which is formed from pro-cambium and vascular cambium. Parenchyma of secondary cortex is formed by cork cambium.

Function of parenchyma:

1. Storage of food is the main function
2. Storage of water especially in xerophytes e.g. Succulents
3. Formation of inter-fasicular cambium and cork cambium in dicot stem
4. Formation of cambium and cork cambium in dicot roots.
5. Formation of wound cambium to heal up wounds.
6. Formation of accessory cambium in monocot stems which exhibit secondary growth
7. Turgid parenchyma provides some sort of mechanical support and maintains shape of plant body.
8. Parenchyma associated with vascular tissue plays an important role in the conduction of sap and translocation of food.
9. In aquatic plants, parenchyma cells store air and provide buoyancy to plants.

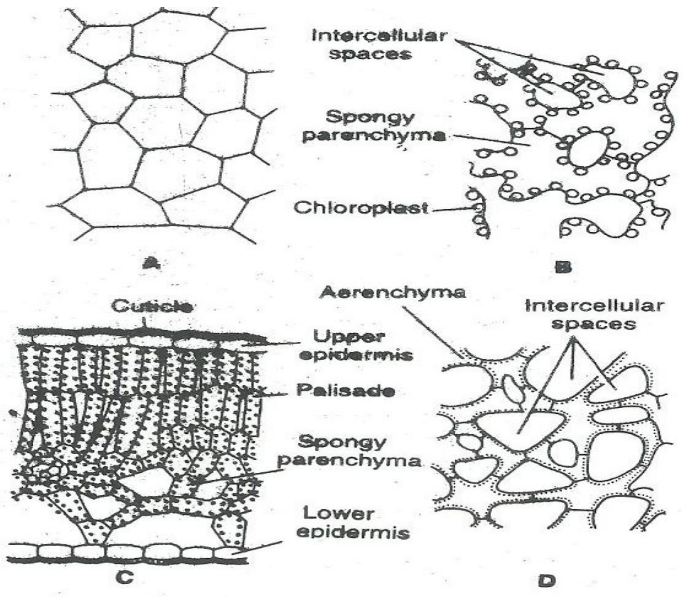


Fig. 2.1, Different types of parenchymatous tissues

A. Parenchyma B. Chlorenchyma C. Palisade and spongy parenchyma D. Aerenchyma

b) **Collenchyma:** The term collenchyma was coined by **Schlieden (1839).** It is the living mechanical tissue which posses considerable tensile strength. It is present beneath the epidermis of young stem, hypodermis of herbaceous (dicot stem), Pedicels of dicot plants, petioles and mid rib of leaves etc. Collenchyma are absent in underground tissues (roots) except in the aerial roots of *Monstera* and leaves & stem of monocots. Peculiarity of collenchyma is uneven thickening which are mostly of pectin in addition to cellulose and hemicellulose. Thickening restricted to corner or certain area. Cells are living and protoplast is highly vacuolated.

Types of Collenchyma: On the basis of thickening on cell wall**, Majumdar** divided collenchyma into three types:

1. **Angular collenchyma:** It is the most common type of collenchyma. Thickenings are confined to corners/angle (where two cell walls come in contact). The cells appear polygonal in cross section. No intercellular spaces are left in them e.g. stems of *Tagetes, Lycopersicon Solanum, Ficus, Vitis, Polygonum, Canabis* etc.
2. **Lacunar/Tubular/Annular collenchyma:** The thickening is confined to the walls of the regions bordering intercellular spaces i.e. thickening around the intercellular spaces. Large intercellular spaces are present e.g. stem of *Cucurbita, Salvia, Malva*, and Petiole of *Malva, Ascelpias*, and aerial roots of *Monstera* etc.
3. **Lamellar collenchyma:** The cells have thickening more on tangential walls than radial walls. Due to such deposition, the cells look like a lamella or plate e.g. stems of *Clerodendron, Raphanus, Helianthus* etc.

Function of collenchyma

1. It is simple living mechanical tissue which provides mechanical strength to growing regions where sclerenchyma is not differentiated.
2. Collenchyma provides elasticity helping plants like *Cucurbita* in bending.
3. Being flexible in nature, it provides tensile strength to the plant body.
4. As the cells of collenchyma are living and often contain chloroplasts, they also take part in photosynthesis.
5. It forms cork cambium in dicot stem during secondary growth.
6. Being located at the margin of the lamina, it prevents tearing of leaves.

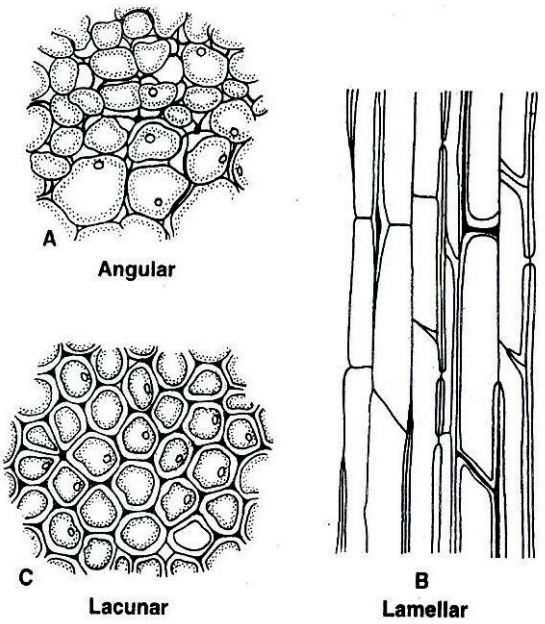


Fig. 2.2, Different types of collenchyma

A. Angular B. Lamellar C. Tubular or lacunar

c) **Sclerenchyma:** Sclerenchyma is also a simple permanent tissue composed of thick walled, dead, often lignified and hard cells. There is a considerable variation in shape, size, origin and development of the cells. The mature sclerenchyma cells differ from parenchyma and collenchyma cells in the presence of lignified secondary walls and the absence of living protoplast. The cells are long, narrow, thick walled, dead and highly lignified. Due to these thickening, the lumen of the cells are greatly reduced with various types of pits. It is the simple dead mechanical tissues occurring in mature organs of plant body. **Mettenius** (1805) discovered and coined the term sclerenchyma.

Types of sclerenchyma: Based on the size and shape, sclerenchyma cells are of two types:

A. Sclerenchymatous fibers

B. Sclereids / Stone cells.

A. Sclerenchymatous fibres: Sclenchymatous fibres are specialized sclerenchyma cells being long, narrow, thick and lignified with pointed or blunt ends. They have great tensile strength, flexibility and elasticity which enable plants parts to withstand a variety of strains and tensions. The fully develop fibres are always dead. Fibres are perhaps the longest cell in the plant kingdom. Generally length of fibre is up to 3 mm but in some cases like Jute (*Corchorus capsularis)*, *Flax (Linum) and Hemp (Cannabis) fibers are upto* 20–550 mm in length. Fibres are always found in sheet. Walls of fibres are lignified, hard and uniformly thickened. Small round or slit like pits are present and intercellular spaces are absent.

Classification of Fibres: Fibres can be classified in two ways i.e. based on their source and economic use and their position in plant bodies.

a) **Fibres on the basis of source and economic use**: There are different types of fibres:

1. **Surface fibres:** Fibres obtained from the seed coat (testa) of cotton and mesocarps of coconut (coir of commerce) are surface fibres. Coir of commerce obtained from the mesocarp and it is called **true fibres**. Cotton fibres are out growth of seed coat i.e. hairs in seed coat hence it not considered as **true fibres**. It is composed of cellulose and not lignin. The former is hard fibres and used in the manufacture of ropes, door mats etc. The later is used in the manufacture of

clothes. Cotton fiber is of two types: (1) **Lint fibres** (long and economically useful) and (2) **Fuzz fibres** (short and not useful).

2. **Bast fibers (Bass fibers):** Fibers obtained from phloem and pericycle is known as bast fibres. They are economically most exploited and are flexible and can be knitted e.g.

a) Jute *(Corchorus species*), Sun hemp *(Crotalaria juncea*) –fibers obtained from phloem used in coarse cloth, ropes, bags etc.

b) Flax or Alsi *(Linum usitatissimum*) – fibers obtained from pericycle used in canvas, linen cloth, high quality writing paper etc.

c) Hemp or Bhang *(Cannabis sativa*) – fibers obtained from pericycle used in formation of ropes, carpets,sacks,

b) Fibers on the basis of their position in the plant body: These are of the following types:

1. **Intra-xylary or wood fibers:** These fibres are associated with xylem and also known as **xylary fibers** and may develop from the same meristematic tissue which gives rise to other xylem elements. They are hard and lack flexibility and hence not useful because can not be knitted. Intra-xylary fibers may sub- divided into **libriform fibers** and **fiber tracheids** on the basis of relative thickness of their walls and the type of pits present on the walls.

i. **Libriform fibers** are characterized by the presence of very thick secondary walls and simple pits. They are long and true fibers.

ii. **Fibers trachieds** are shorter, less thick with bordered pits.

2. **Extra-xylary fibres:** These fibres which are also called **bast fibres** are found in different tissues out side the xylem such as cortex (cortical fibres), pericycle (pericycle fibres) or even phloem (phloic or phloem fibres). The bast fibres are long and spindles shaped with tapering ends and are usually un-branched. Some time they are very long as in hemp and flax and are obtained from pericycle and are of great economic value. They have thick walls with simple or bordered pits. The thickening is either of lignin or of cellulose. Alternate layers of lignin and cellulose have also been fond in the wall layers of some bast fibres. Pericycle fibres are also called **perivascular fibres** and phloem fibres are generally called **bast fibres**.

Origin of fibers: They may either originate from pro-cambium, or the cambium or from the ground meristem. The former is present in vascular tissue (xylem and phloem), where as the later occur in the centre.

Functions of the fibres: Chief function of the fibre is to give mechanical support to the plants and their position and distribution in the plant save the plants from various stresses and strains e.g strong wind.

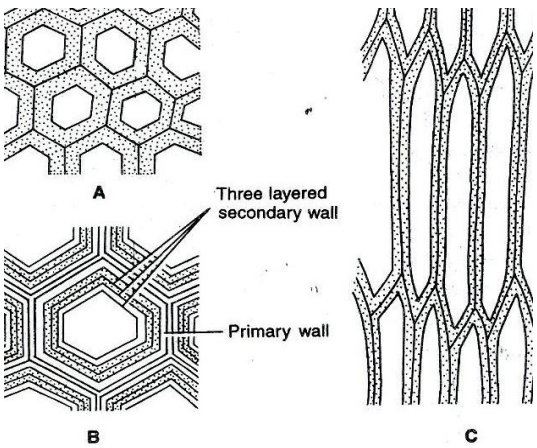


Fig. 2.3, Sclerenchyma A. and B. T.S. C. L.S.

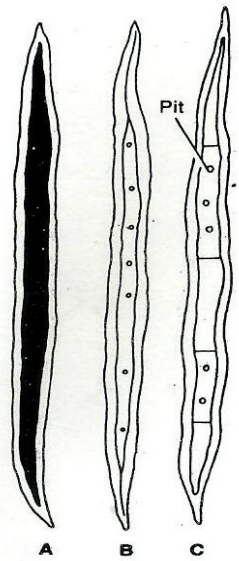


Fig. 2.4, A.-C. Sclerenchymatous fibres

B. Sclereids: The term Sclereids given by **Tschierch (1885).** It is often called stone cells or sclerotic cells. They are widely distributed and may occur almost any where in the plant body either singly or in group. They may be isodiametric, spherical, oval, stellate, T-shaped etc. They are dead cells with small lumen. Their walls are hard, usually possessing highly thickened secondary walls with lignin and some time suberized thick secondary walls & simple pits. Sclereids are most abundant in soft tissue like cortex, phloem, medulla, fleshy fruits, seed coats and fruit walls.

Classification of sclereids: Tschierch classified the sclereids into following categories on the basis of their shapes.

1. **Brachysclereids** or **grit cells** or **stone cells:** These are short and roughly isodiametric resembling parenchyma cell in their shape. These occur in the bark, cortex, pith *(Nicotiana)* and phloem of stem of *Cinnamomum* and fruit pulp of *Pyrus, guava, annona* etc. Grittiness in the pulp is due to these cells only. They are found in the endocarp of drupe fruits, so that endocarp become hard e.g. coconut, mango, almond, etc.
2. **Macrosclereids:** These are rod shaped, elongated and columnar sclereids, occurring in the seeds and fruits in the form of layers. In Leguminous seeds, it is present in seed coats. Due to their presence, seed coat become hard and is the cause of dormancy in leguminous seeds e.g. *Phaseolus, Pisum* etc. They are also known as malphigian cells. Malphigian cells form palisade like layer in the outer seed coat of legumes and epidermis of onion. Seed coat of French bean is hardest among the leguminous plants. Seed coat of Lotus is hardest (stony) and may remain viable for more than 100 years.
3. **Osteosclereids:** These cells are bone or pillar shaped, both ends are almost dilated and are found in the leaves of *Hakea and Osmanthus fragrans* etc.
4. **Astrosclereids:** They are star shaped, found in the leaves and petioles of *Nymphea*, leaves and stem of *Thea* (tea) and *Trochodendron* and also in the leaves of *Victoria, Lotus* etc.
5. **Trichosclereids (filiform sclereids):** They are hair like sclereids, occasionally branched, occur in intercellular spaces in the leaves and stem of certain hydrophytes (floating leaves) e.g. *Victoria, Nelumbo, Nymphea, Olea* and in the cortex of aerial roots of *Monstera*.

Origin of sclereids: Sclereids usually originated from parenchymatous cells. At maturity secondary wall is laid down and then become very thick and later on cytoplasm and nucleus disappear so that the sclereids are dead cells.

Functions of sclerenchyma:

1. Sclerenchyma provides mechanical strength.
2. Sclerenchyma saves the plant from various stresses and strains (e.g. bending, shearing, compression, pull etc.) caused by environmental forces like strong wind.
3. It presence in leaf provides it rigidity and prevents it from collapsing.
4. Sclereids give strength to seed covering.
5. Sclerenchyma helps in dehiscence of many fruits (e.g. pods) due to differential distribution of sclerenchyma fibers.

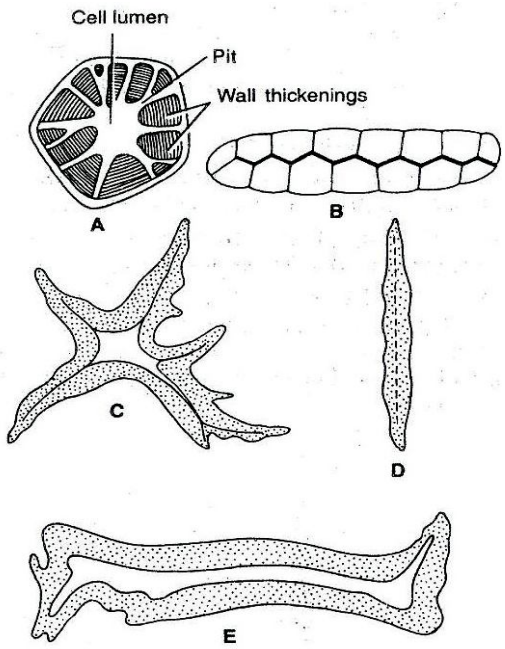


Fig. 2.5, Types of stone cells or sclereids

A. Brachysclereids B. Macrosclereids C. Aestrosclereids D. Filiform E. Osteosclereids

Difference between parenchyma, collenchyma and sclerenchyma

Characters	Parenchyma	Collenchyma	Sclerenchyma
Definition	Simple, living storage tissue.	Simple, living, mechanical tissue.	Simple, dead mechanical tissue.
Shape of the cells	Spherical or oval, sometimes rectangular or irregular.	Elongated, polygonal or spherical or oval,	Elongated, polygonal or short and irregular.
Arrangement of cells	Loosely arranged with intercellular spaces. Compactly arranged in epidermis, endodermis and pericycle.	Either loosely arranged or compactly arranged.	Compactly arranged without intercellular spaces.
Cell wall	Thin, has only primary wall (cellulose),	On evenly thick, has a primary wall (cellulose and a secondary wall (hemi cellulose or pectin).	Uniformly thick) made up of a primary wall (cellulose) and a secondary wall (lignin).
Cytoplasm	Abundant, granular.	Reduced, granular.	Replaced by lignin.

Characters	Parenchyma	Collenchyma	Sclerenchyma
Nucleus	Large, prominent just above the vacuole	Large and prominent just above the vacuole.	Absent at maturity
Cell organelles	Present in a highly functional state.	Usually present in a functional state,	Absent at maturity.
Vacuole	Present, large and prominent,	Present, large and prominent,	Represented by a lumen.
Ergastic substances	Always present, usually in the form of reserve food.	Usually present in the form of secretary products.	Absent
Functions	Storage, respiration photosynthesis, absorption secretion, buoyancy and protection.	Mechanical support, sometimes photosynthesis and storage.	Mechanical support and protection.
Occurrence	In all the regions of the plant body, forms the ground tissue and epidermis.	In the shoot system only. Absent in the root.	In all the regions of plant body particularly in fruit wall and seed coat.

Complex Permanent Tissue: A complex tissue is made up of more than one type of cells, functioning together as a unit or in other words the complex permanent tissues are those whose cells are heterogenous in nature i.e. cells having dissimilar origin and structure but performing a common function. They are also called vascular tissue and they are xylem and phloem. Xylem and phloem are together called conducting tissues and are organized into vascular bundles. It is also called physio-mechanical tissue.

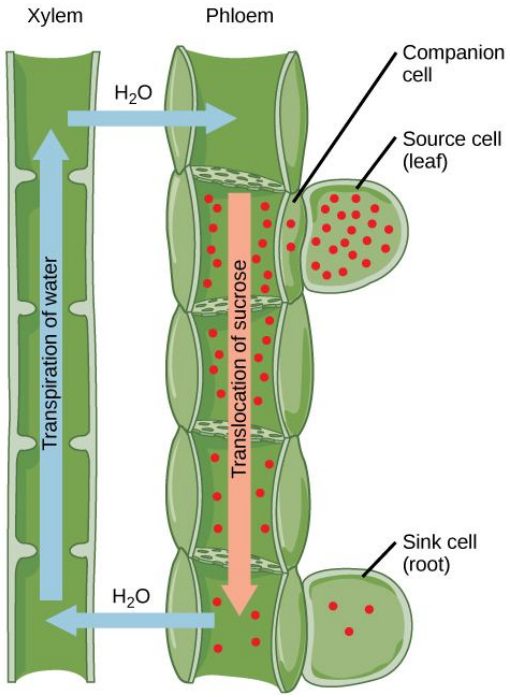


Fig. 2.6, Showing the basic function and interrelationship of two complex tissues xylem and phloem

Xylem (Wood or Hadrome): Xylem is composed of living as well as dead cells. It is primarily concerned with the conduction of water and minerals and also provides mechanical support to the plant. As a conducting strand, xylem forms a continuous channel through roots, stems, leaves, flower

and fruits. The xylem elements that develop first from the pro-cambial strands are called proto-xylem elements, and those develop later form the meta-xylem elements. The term xylem was coined by Nageli (1858) and the term Hadrome (for vascular part of xylem i.e. vessels and tracheids) was coined by Haberlandt (1914).

Component of xylem: There are mainly three components of xylem: Tracheary element (trachieds and vessels), xylem parenchyma and xylem fibres.

1. **Tracheary Elements:** Tracheary elements are highly specialized cells, principally concerned with the conduction of water. Two kinds of tracheary elements occurring in xylem are tracheids and vessels. Both are dead cells and devoid of protoplasm at maturity. They are more or less elongated cells with lignified secondary walls. Two kinds of tracheary elements are as follows:

a. **Tracheids:** Term tracheid was given by Sanio. Tracheids constitute 90-95% of wood (xylem) in gymnosperms and hardly 5% of wood in angiosperms. Trachieds are long, slender cells with overlapping tapered ends. Tracheids are the more primitive (less specialized) of the two xylem cells. They are found in most woody, non-flowering plants. In primary xylem, they originate from the cells of pro-cambial strands by lignifications of their walls. The cells are joining together from their ends to form long rows which extend from roots via stem to leaves. Transverse septum is present between two tracheid cells which bear pits. The deposition of lignin on the cell wall is responsible for different types of thickening. It is annular and spiral in first formed xylem (proto-xylem) and scalariform, reticulate and pitted in later formed xylem (meta-xylem). Water moves between tracheids cells via the bordered pits. Bordered pits are thin areas in the cell walls where only primary cell wall material has been deposited.

Tracheids are present in all vascular plants, whereas vessels are confined to angiosperms. However in some primitive angiosperms, the vessels are absent e.g. family Winteracaeae (*Wintera*), Tetracentraceae (*Tetracentron*) and Trchodendraceae (*Trochodendron*).

b. **Vessels or Tracheae:** Vessels is long, cylindrical, tube like structure with lignified walls and a wide central cavity. Vessels are arranged in longitudinal series in which the transverse wall (the end plates) is perforated and as such the entire structure is looks like a water pipe. Their end plates are partially or wholly dissolved (transverse septum is absent between two cells) allowing them to form long, hollow tubes up to 3 meters long. Vessels are the example of dead Syncyte (Syncyte: Cell which is formed by fusion of cells) arising through the dissolution or re-sorption of the end walls. When the end walls are completely dissolved and only one pore is present. This condition is called simple perforation plate. When the end walls remain intact but consist of a number of pores; the condition is called multiple perforation plate (e.g. *Lepidodendron, Magnolia*). Perforation plate, therefore, is the region where the vessels elements are connected with each other. The larger diameter and lack of end walls allow vessel elements to transport water more rapidly. Vessel elements are evolutionarily more advanced than tracheids. They are found in angiosperms and are one of the major reasons why angiosperms are the dominant land plant. Thickening of the cell wall is same as in tracheids but the metaxylem generally possesses simple pits. Lumen is wider than tracheids. Due to absence of transverse septum, vessels work as a pipe line during conduction of water. Vessels can be derived from the tracheids (1) by the dissolution of pit membranes of pits which make the end wall perforated or (2) by the dissolution of entire walls. The length of vessels rarely exceeds 10 cm; though attain the length of two meters as in *Quercus,* and 3-6 meters as in *Wistaria* and *Eucalyptus*. Some primitive angiosperms which lack vessel are: e.g. *Wintera* (winteraceae), *Tetracentron* (Tetracentraceae) and *Trochodendron* (Trochodendraceae). Vessels are also absent in stems and leaves of *Yucca* and *Draceaena* of monocots. *Gnetum, Ephedra* and *Welwitschia* are some advanced gymnosperms which bear vessels. Vessels are also present in some pteridophytes e.g. *Selaginaella, Equisetum Pteridium* (not in all species of *Selaginella, Equisetum* and *Pteridium).*

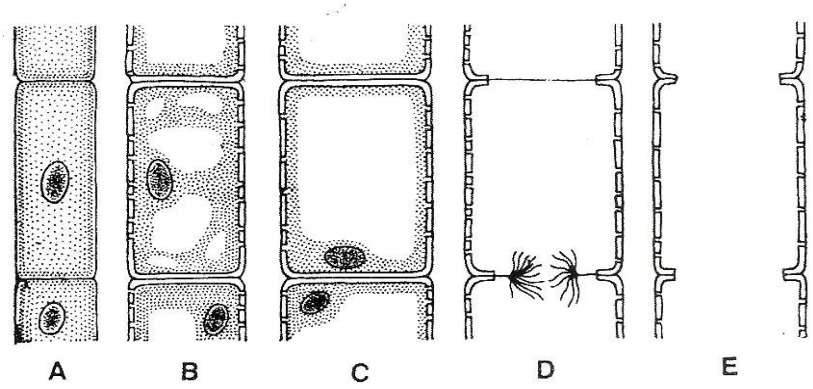


Fig. 2.7, Stages in development of tracheae

Functions of Tracheary Elements: They serve as the efficient mode of transport of water and minerals. However, vessels are more efficient than tracheids due to presence perforation plates. They also give mechanical support to the plant body.

2. **Xylem (Wood) Parenchyma:** Parenchyma associated with xylem is called xylem parenchyma or wood parenchyma. Xylem parenchyma is the only living component of xylem. It is made of parenchymatous cells with thin wall and living contents. The walls have cellulose. It is thin in primary xylem and thick in secondary xylem. In the secondary xylem, the wall is slightly lignified. Another type of parenchymatous cells is also present in secondary xylem. These are ray parenchyma cells. It helps in lateral conduction of water and storage of foods. The distribution of xylem parenchyma in angiospermous wood is variable and its two main categories are recognized:

a) **Apotracheal Type:** In this case the parenchyma cells are not in contact with the xylem vessels.

b) **Paratracheal Type:** In this case the parenchyma cells are always associated with the vessels.

3. **Xylem (Wood) Fibres:** Xylem or wood fibres are sclerenchymatous dead cells having extremely lignified thick walls with narrow lumen and usually pointed at both ends. It is present both in primary and secondary xylem. The secondary xylem contains abundant xylem fibres which greatly contribute in its mechanical strength. Xylem fibres are derived from tracheids. Xylem fibres are of two types:

a) **Fibre tracheids:** The fibres that are in intermediate stage between fibres and tracheids possess thin walls and border pits are called **fibre tracheids**.

b) **Libriform fibres:** The fully developed xylem fibres posses only a few simple pits and a reduced lumen are called **libriform fibres** or **typical fibres**.

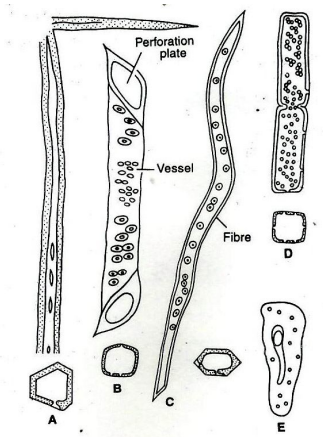


Fig. 2.8, Xylem elements A. Tracheid B. Vessel C. Fibre D.-E. Parenchyma

Transfer Cells: Beside the above mentioned cell types, yet another type of cells are associated with xylem which are called **transfer cells**. These cells have dense cytoplasmic contents, large nuclei, numerous mitochondria and cytoplasmic reticulum. They are characterized by wall in growth of non-lignified secondary wall. Plasmodesmata are also present on the walls of the two adjacent transfer cells. Transfer cells are associated with the internal transfer of solutes.

Difference between Vessels and Tracheids

S. No.	Vessels (Tracheae)	Tracheids
1.	Longer	Comparatively short.
2.	Usually up to 10 cm long.	Usually up to 1 mm long.
3.	Compose of row of cells placed one above the other.	A tracheid is a single cell.
4.	Intervening wall between the two cells absent hence composed to pipes.	Nothing like this.
5.	Since formed by the fusion of row of cells, it is called a syncyte.	No fusion of any cells. End walls of the tracheids placed one above the other are separated by cross walls.
6.	Bordered pits absent.	Bordered pits present in cross walls.

Proto-xylem and Meta-xylem: On the basis of the time of origin, the xylem is classified as of two types: proto-xylem and meta-xylem. The xylem that develops first from the pro-cambial strands is called **proto-xylem.** Proto-xylem elements consist of smaller tracheids and vessels possessing annular or spiral thickenings. However, the xylem that develops in the later stage is referred to as **meta-xylem**. Meta-xylem elements consist of bigger tracheids and vessels possessing scalariform, reticulate or pitted thickenings.

Difference Between Protoxylem and Metaxylem

S.No.	Protoxylem	Metaxylem
1.	It is represented by vessels that are formed earlier.	It is represented by vessels that are formed later.
2.	Lumen is narrow.	Lumen is wider.
3.	Vessels exhibit annular and spiral type of thickening.	Vessels exhibit scalariform, reticulate and pitted type of thickening.

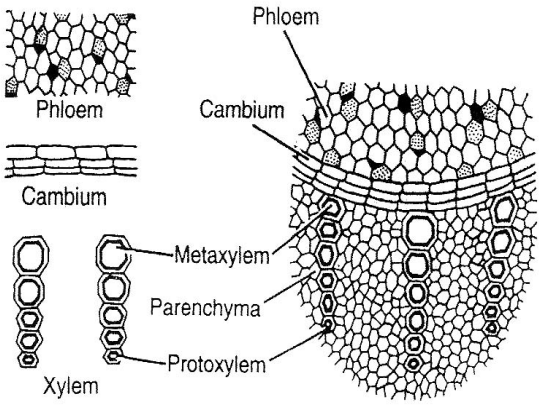


Fig. 2.9, Parts of a vascular bundle

Types of xylem: Depending upon the position of proto-xylem with respect to meta-xylem in vascular bundles, the xylem can be further classified as of three types:

1. **Exarch:** Proto-xylem lying outside the metaxylem i.e. towards periphery e.g. roots.

2. **Mesarch:** Protoxylem lying in the middle of meta-xylem e.g. stem of ferns
3. **Endarch:** Proto-xylem lying inside the meta-xylem i.e. towards centre e.g. stem of gymnosperms and angiosperms

Development of water conducting elements: Tracheids and vessels collectively known as water conducting elements and there are three ways of development of these conducting elements:

1. **Centrifugal:** In this type of development, the proto-xylem formed towards the centre and meta-xylem formed away from the centre towards the periphery. This condition is known as endarch e.g. stem of gymnosperms and angiosperms.
2. **Centripetal:** In this type of development, proto-xylem is formed away from the centre near the pericyle and meta-xylem is formed towards the centre. This condition is called exarch e.g. roots.
3. **Centripetal and centrifugal:** In this condition, meta-xylem is formed from both sides of the elements of proto-xylem. So, that proto-xylem is surrounded by meta-xylem. This condition is known as mesarch.

Types of Thickening

a) **Annular:** Wall thickening is in the form of a ring.

b) **Spiral or helical:** Wall thickenings are like spiral or helix.

c) **Scalariform:** Wall thickenings look like ladder because the spiral or helical bands are joined in certain regions giving the appearance of a ladder.

d) **Reticulate:** Wall thickenings are in the form of a network.

e) **Pitted:** Entire wall surface is uniformly thickened except for small un-thickened areas called pits. Pitted tracheids show the most advanced type of thickening. Pits appear circular, oval or angular in surface view.

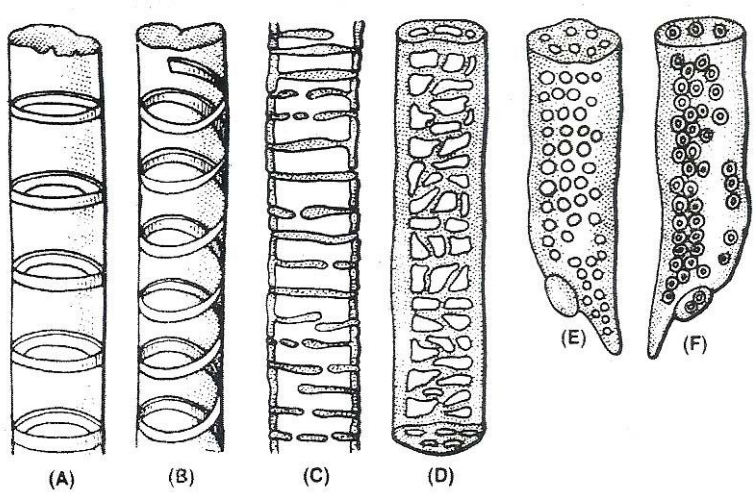


Fig. 2.10, Lignified thickening in xylem tracheids and tracheae

A. Annular , B. Spiral, C. Scalariform, D. Reticulate, E. Simple pit, F. Bordered pit

Pits: During the secondary thickening of lignin, entire wall surface is uniformly thickened except for small un-thickened areas called pits. Pitted tracheids show the most advanced type of thickening. Pits appear circular, oval or angular in surface view.

Types of pits: There are two types of pits; simple pits and bordered pits

1. **Simple pits:** Simple pit pairs occur in parenchyma cells, in medullary rays, in phloem fibers, companion cells, and in tracheids of several flowering plants. In the simple pits, the pit cavity remains of the same diameter and the pit or closing membrane also remains simple and uniform

in its structure. The simple pit may be circular, oval, polygonal, elongated or somewhat irregular in its facial view. The simple pits occurring in the thin walls are shallow, whereas in thick wall the pit cavity may have the form of a canal passing from the lumen of the cell towards the closing or common pit membrane. The diffusion of protoplasm takes place through these pits.

2. **Bordered pits:** They are abundantly found in the vessels of many angiosperms and in the tracheids of many conifers. They are more complex and variable in their structure than simple pits. The overarching secondary wall which encloses a part of the pit cavity is called **pit border**, which opens outside by a small rounded mouth known as **pit aperture**. The overarching rim forms a border around the aperture and thus named **'bordered pits'**. The pit aperture may be of various shapes in the facial view. It may be circular, lenticular, linear or oval. The space between the closing membrane and the pit aperture may be called the pit chamber and the canal leading from pit chamber to the lumen of the cell may be termed as pit canal. The closing membrane of a bordered pit pair which consists of the parts of two primary walls and the intercellular substance or middle lamella, is somewhat thickened in its central part. This thickening is called torus which remains surrounded by a delicate margin. In many bordered pits, the closing membrane may change its position within pit cavities. The torus may remain in central position or it may shift to the lateral position. As the torus is shifted to the lateral position the pit aperture closes, and the passage of the protoplasm may take place only by diffusion through torus.

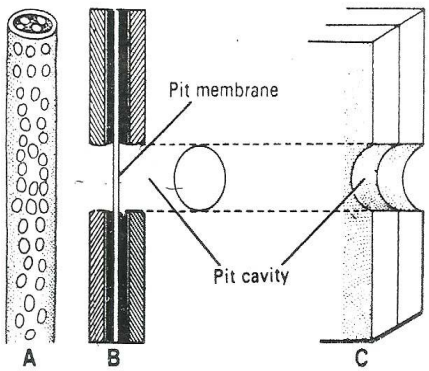


Fig. 2.11, Structure of simple pit

A. Cell wall from front, **B**. L.S. of A, **C.** L.S. of pit

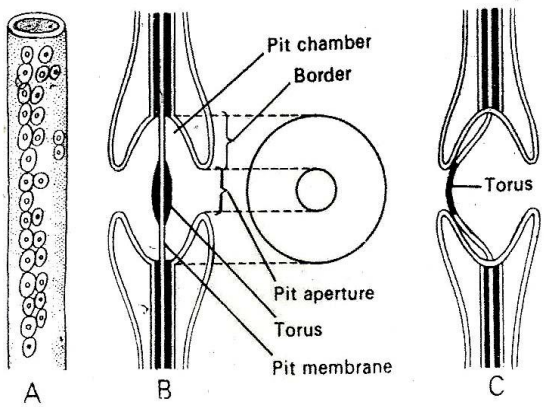


Fig. 2.12, Structure of bordered pit

A. Cell wall from front, **B**. L.S. of A, **C.** L.S. of bordered pit

Phloem: Term phloem was given by **Nageli (1858).** It is mainly responsible for translocation of foods (organic material from one place to another place). The term **leptome** for phloem was given by **Haberlandt (1914).** Actually the leptome term was for sieve element (vascular part of phloem) only.

On the basis of development, the phloem is classified into two categories i.e. primary and secondary phloem. Primary phloem is derived from pro-cambium and secondary phloem derived from vascular cambium. First formed phloem is called **proto-phloem** and later formed phloem is called **meta-phloem**. Phloem remains active for less duration as compared to xylem. Phloem is also called **bast** because phloem fibers of some plants are used for binding purposes e.g. *Flax* and *Hemp*.

Components of Phloem: Phloem consists of four kinds of elements (cells):

a) Sieve tube elements,

b) Companion cells

c) Phloem parenchyma

d) Phloem fibers

a) **Sieve tube elements (Sieve cells & sieve tubes):** The translocating elements of phloem (sieve cells and tubes) are collectively known as **sieve elements**. A sieve cell was discovered by **Hartig.** Sieve cells are elongated with tapering end arranged in parallel groups. These are primitive type of sieve element. **In angiosperms**, the sieve cells are arranged one above the other with oblique or transverse partition wall with many perforations which gives the appearance of sieve (The partition wall is called sieve plate and the perforation is called sieve area). In this way, in angiosperm, sieve cells form a tube like structure and now it is called as **sieve tubes**. Sieve plates are called **simple sieve plate** when they consist of only one sieve pore (e.g. *Cucurbita, Nicotiana*). A sieve plate with many sieve pores is called **compound sieve plate** (e.g. *Vitis*). Sieve plate is present between two sieve cells. It is porous and material only transport through these pores. In pteridophytes and gymnosperm, sieve cells do not form sieve plate and arranged irregularly and sieve cells have sieve plate on their lateral walls. Thus the conduction of food is in zig-zag manner. Sieve cells are living, thin walled and cellulosic**.** Sieve tube is the example of **living syncyte.** Although the sieve cells are living but lack nucleus at maturity and nucleus of companion cells control its functional activities. Central vacuole present in each sieve cell. During unfavorable condition (winter), the cytoplasmic strands are get plugged (en-sheathed) by the **callose** in the sieve area and transport of food material is reduced. This is called **callose pad**. The sieve plate is protected by callose pad. It is also prevented from bacterial infection and drought. The callose material dissolved in summer or spring and transport of food restored. Mature sieve tubes always remain closed by callose. Callose is a **polysaccharide (B-1-3 glucan),** it stains **blue with aniline** and forms glucose on hydrolysis. Callose pad deposited on sieve areas for only limited unfavorable period is called **seasonal callose,** while that formed permanently in functionless old sieve tube is called **definitive callose.** Sieve cells contain special type of protein in the form of slime layer (slime bodies) which gets aggregated in the form of tubules called **P-protein**. P. protein repair damaged sieve cells.

b) **Companion Cells:** It is highly specialized parenchyma, which is present in the phloem of angiosperms and absent in pteridophytes and gymnosperms**.** It is thin walled elongated cell. In primary phloem, normally one companion cell is associated with each sieve cells which maintains the cytoplasmic connection with each other through simple pits (plasmodesmata). In secondary phloem of woody plants, many short companion cells are associated with each sieve tube members. Each companion cell is living; contain dense cytoplasm and a large elongated nucleus. Actually the mature sieve cells lack nucleus and its cytoplasm controlled by the nucleus of companion cells. Companion cells are absent in ***Austrobaileya*** (an angiosperm). A special protein rich cell called **albuminous cell** associated with sieve cell in place of companion cells is found in **conifers (gymnosperms)** e.g. ***Pinus*.** Albuminous cells are analogous to companion cells of angiosperms. Companion cells are present in Gymnosperms like ***Gnetum, Ephedra and Welwitschia.*** Companion cells and sieve tube are the sister cells i.e. related ontogenetically as they are formed from the same mother cell. Companion cells, in association with phloem parenchyma, play an important role in the maintenance of pressure gradient in the sieve tubes. They form a link between sieve tubes and other cells and control the passage of materials.

c) **Phloem Parenchyma:** These are living parenchymatous cells which may be cylindrical, sub-spherical or polyhedral in shape. The cells have dense cytoplasm and prominent nucleus. The cell wall is composed of cellulose. They are also called bast parenchyma. Its main function is storage of food and conduction of food in radial direction. It is absent in most of the monocots and also absent in Ranunculaceae (dicot). In primary phloem, the cells of phloem parenchyma are longitudinally oriented. In the secondary phloem, they are of two types: (i) those elongated vertically and (ii) those elongated radially (called secondary phloem ray cells).

d) **Phloem Fibers:** They are commonly known as **bast fibers or bass** and are the main commercially important fibers. They occur both in primary phloem and secondary phloem. These are much elongated, usually unbranched and have pointed needle like apices. This is the only dead cells in the phloem which is lignified. These fibers have only simple pits. The fibers provide mechanical support to the sieve elements. They are of considerable economic important in some plants e.g. Jute, Hemp, Flax etc. they are used for making ropes, rough clothes and mats etc.

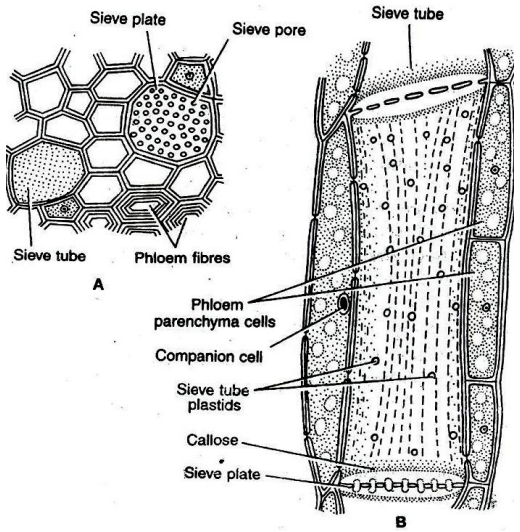


Fig. 2.13, A. T.S. of phloem showing its elements, B. L.S. of phloem

Transfer cells: These are recently reported cells in phloem tissue and are considered responsible for short distance transport of metabolites. These cells are characterized by having wall ingrowths and a cytoplasm rich in organelles. Transfer cells particularly common in minor leaf veins and at nodal region of the shoots. They have specialized type of non-lignified secondary cell wall deposition on the inner side of the primary wall. Plasmodesmata usually occur between the two adjacent transfer cells.

Difference Between Sieve Tube and Sieve Cells

S. No.	Sieve Tube	Sieve Cells
1.	They posses well differentiated sieve areas.	Sieve areas are less specialized.
2.	Sieve areas are restricted to sieve plates which are usually the cross walls that may be transversely placed or oblique.	Sieve areas do not form sieve plates and may be scattered all along the radial walls or even end walls.
3.	They are found in angiosperms.	They are found in pteridophytes and gymnosperms.
4.	They have always companion cells.	The companion cells are absent.

Internal phloem (intraxylary phloem) and included phloem (internal phloem):

Normally, the phloem is present on the outer border of xylem so that they lay one above the other. But sometimes, the phloem deviates from it normal arrangement. In some dicot families, the primary phloem strands occur in small groups internal to the xylem in the pith; this phloem strand is called **internal phloem** (e.g. Apocynaceae, Solanaceae, Convolvulaceae, Ascelepiadaceae etc.). Some time groups of secondary phloem are present scattered among the secondary xylem; this phloem strand is called **included phloem** (e.g. Amaranthaceae, Chenopodiaceae and Nyctaginaceae).

Note: Internal phloem is primary in origin and included phloem is secondary in origin.

Chapter-3

Special Tissues or Secretory Tissues

Special tissues are the group of cells concerned with the secretion of material like resins, mucilage, latex, oil etc. They are also called **secretory tissues**. These tissues are of two types:

A. **Glandualr tissues**

B. **Lacticiferous tissues**

A. **Glandular tissues:** Glandular tissues are consisting of glands and glands are specialized group of cells. A gland, however, is defined as a specialized group of cells that are rich in capacity to excrete or secrete products. The products are released by cytoplasm of the cell and may be stored in their vacuoles or in central cavity.

Types of glandular tissues: Glandular tissues are of two types:

a) External glands

b) Internal glands

a) **External Glands:** External glands are epidermal in origin and are present on epidermis or surface of stem or leaves. They may be of the following types:

1. **Glandular hairs:** Glandular hairs occur in the epidermal layers of leaves and may be unicellular or multicellular. They are of different kinds e.g. stinging hairs present in *Utrica dioca.* Here unicellular, brittle hairs are present on under surface of leaves. At its base a gland is present which secrete poisonous substance **(Formic acids)** which causes a lot of irritation resulting in blisters on the skin. They are also known as **spiny glands**.

2. **Nectaries or nectar secreting glands:** Nectaries help in pollination and generally found on floral parts (exception is *Passiflora* where nectaries are present on leaves). Floral nectaries occur on different parts of the flower e.g. nectaries present on petal (*Ranunculus*), nectaries present below ovary in the form of disc (member of Rutaceae), nectaries present at the base of stamen (*Brassica compestris*) etc.

3. **Digestive glands or enzymes secreting glands:** These are present in insectivorous plants and produce proteolytic enzymes which help in digesting proteins from the body of insects e.g. *Drosera* (sundew), *Nepenthes* (pitcher plant), *Utricularia* and *Dionaea*. These insectivorous plants compensate their deficiency of nitrogen as they grow in nitrogen deficient soil and by capturing insects; they fulfill their needs of nitrogen.

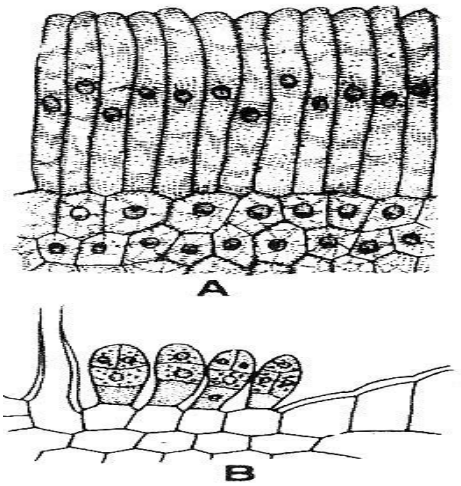


Fig. 3.1, Nector secreting glands

A. *Euphorbia*, **B**. *Vicia*

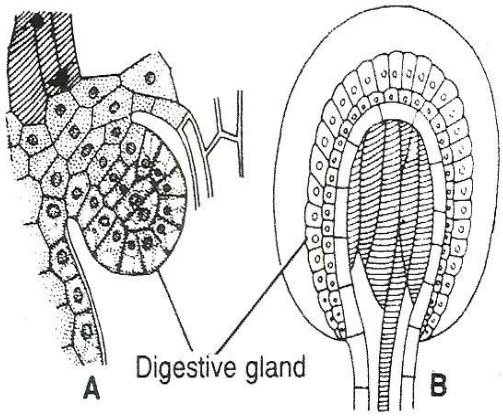


Fig. 3.2, Digestive glands

A. Nepenthes, **B.** Drosera

b) **Internal glands:** These glands are spherical or tubular and found inside the plants. These are of several types:

1. **Oil glands:** In some plants the internal glands secrete a volatile oils (essential oils) into central reservoir e.g. oil glands present in fruit walls (*Citrus*), oil glands present in leaves *(Eucalyptus)*. They are lysigenous in origin. The lysigenous cavity is formed due to dissolution of glandular cells.

2. **Resin/Tannin/Gum glands or canals:** Resin canals are present in *Pinus, Agathis* and *Cedrus* etc. They are schizogenous in origin. It is formed due to break down of glandular cells. Some time it becomes balloon like and called **Tylosoids.** Tanin, resin, mucilage canals, gum secreting glands are the example of internal glands. Maximum resin glands are present in *Palm*. Gum glands are found in *Acaccia* (Babool) etc.

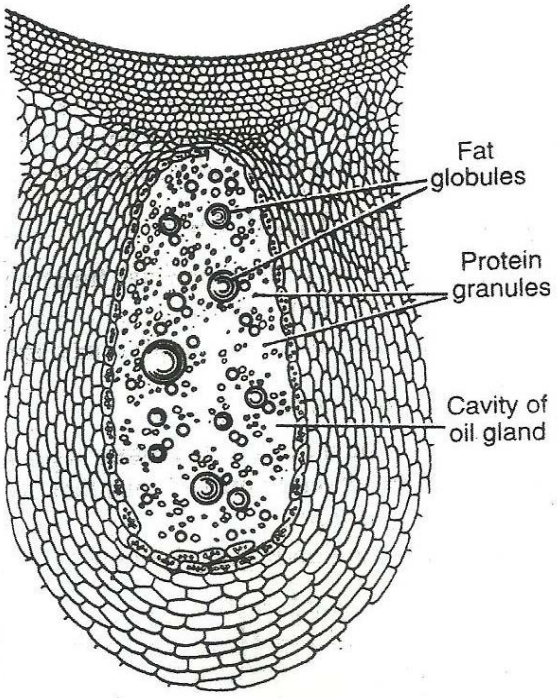


Fig. 3.3, T.S. of oil secreting gland in the rind of *Citrus*

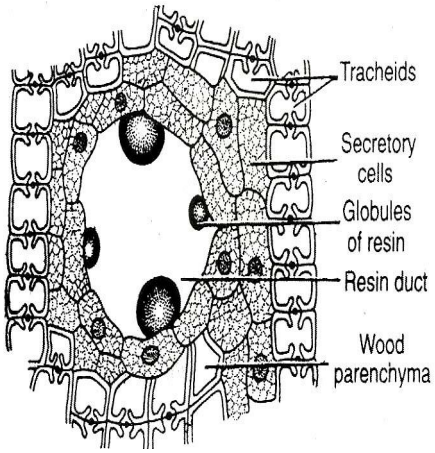


Fig. 3.4, Resin secreting gland of *Pinus*

3. **Hydathodes (Water secreting glands or water stomata):** They are the water exuding structures of plants inhabiting the humid tropics. They are the cause of guttation. These hydathodes are found in the leaves of many angiosperms and are located on leaf margin (e.g. *Tropaeoleum*) or at the tip of leaves (e.g. *Colocasia*). The cells of mesophyll adjacent to the vascular bundle proliferate to give rise to epithem. The cells of epithem are thin walled, elongated with dense cytoplasm and deficient in chloroplasts. They have a well develop system of intercellular spaces and are in close contact with terminal tracheary elements. Overlapping the epithem are present two guard cells. The water that moves out of the tracheids under condition of high root pressure and humidity is ultimately discharged through the terminal pore of the hydathode.

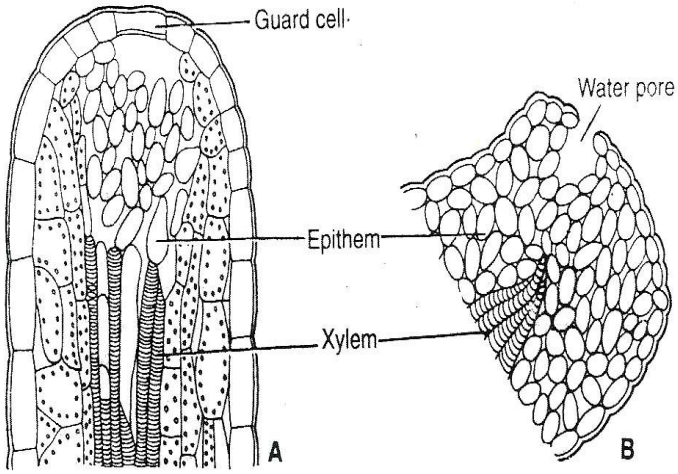


Fig. 3.5, L.S. of water secreting gland

A. *Primula*, **B.** Tomato

B. **Lacticiferous tissue:** These are the specialized parenchyma cells which secrete milky juices called latex. Latex is the milk of plants. These tissues present through the ground tissues and contain organic substances of much importance like wax, resin, proteins, essential oils and sugars, starch granules etc. Latex producing plants are called **petrocrops.** Starch granules present in latex are **dumb bell shaped.** On the basis of their structure and development, the following types of laticifers are recognized: Latex cells & Latex vessels

1. **Latex cells:** These are also called **non-articulated lacticifers**. These are made up of individual cells. They are multinucleate cells of immeasurable length. They develop from single cell which

are capable of growing independently. Such cells grow more rapidly than the neighboring cells and get branched also e.g. *Calotropis, Nerium, Euphorbia, Cannabis, Vinca minor, Utrica dioica, Catharanthus roseus, Ficus etc. (family Ascelpidaceae, Euphorbiaceae, Apocynaceae & Moraceae)* contain latex cells.

2. **Latex vessels:** These are also called **articulated lactcifers** because it is formed due to union of latex cells. (Latex cells Joined together by dissolution of septa) e.g. *Papaver, Argemone* (yellow latex), *Banana*, sunflower, *Sequoia* (red latex). Highly developed lactcifer vessels occur in fruit wall of *Opium*. Latex of *Carica papaya* (Papaya) contains the enzyme Papain. Chicle (chewing gum) obtained from latex of *Achras zapota.* Though ***Hevea brasiliensis*** **(para rubber)** belong to family **Euphorbiaceae,** these contain latex vessels (not latex cells). Para rubber and Indian rubber are obtained from the latex of *Hevea brasiliensis* and *Ficus elastica* respectively. Latex of Poppy *(Papaver somniferum*) yields opium which contains the alkaloid **morphine.**

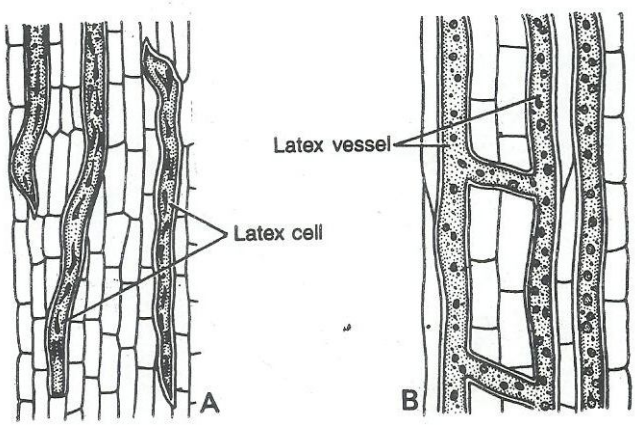


Fig. 3.6, L.S. of Lacticiferous tissue

A. Latex cells B. Latex vessel

Chapter-4

Tissue System

All the tissue of plant which performs the same function regardless of position in the body constitutes the tissue system. According to function, tissue systems are of five type's i.e. protective, mechanical, photosynthetic, fundamentals and vascular tissue system. Sachs (1875) classified the tissue based on their position and morphology into three types: Epidermal (Dermal) tissue system, ground (fundamental) tissue system and vascular (conducting) tissue system.

(A) Epidermal Tissue System: The epidermal tissue system makes up the outside covering of the plant. It is in direct contact with the external environment. This system comprises the followings:

a. **Epidermis:** The epidermis consists of a single layer of cells that covers the majority of young plants. The epidermis is present throughout the life of plants that exhibit only primary growth. It derived from protoderm (dermatogen). It is generally single layered (uniseriate) but multilayered (multiseriate) epidermis is present in the leaves of *Nerium, Ficus, Pepromia* etc. Its cells are compactly arranged without any intercellular spaces. Epidermal cells are of varying shape and size and form a continuous layer which is interrupted by stomata. They are mostly tubular and appear flattened and rectangular in a cross section. In some cases, they are isodiametric. The outer tangential wall is generally thickened due to presence of cuticle, wax, resin etc. The radial walls are generally thick above and gradually decrease inwards. Thin cuticles are present in mesophytes, thick cuticle in xerophytes and no cuticle is present in hydrophytes. Epidermal cells lack chloroplast except in some ferns, sub-merged hydrophytes and guard cells.

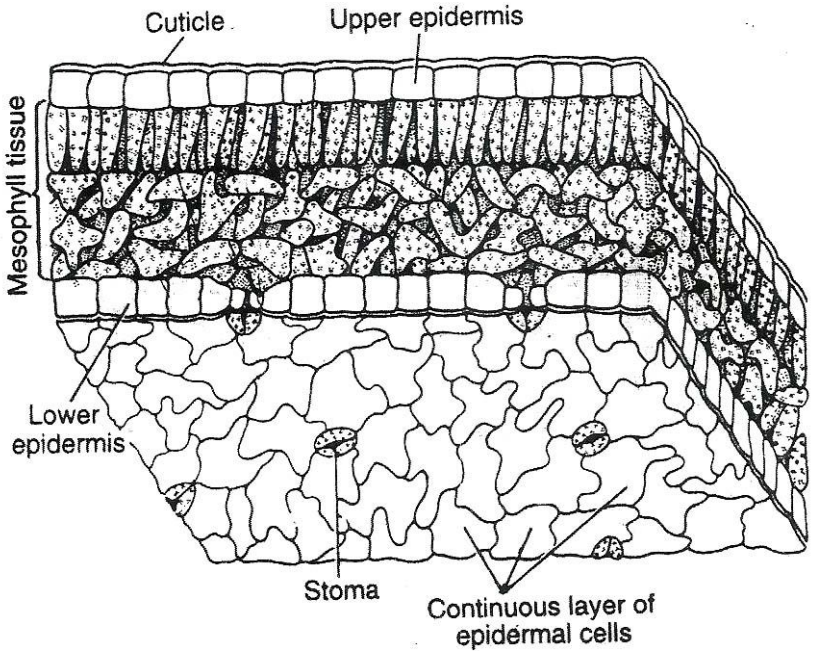


Fig. 4.1, Three dimensional structure of leaf showing epidermal tissue system

Specialized epidermal cells: Some epidermal cells are specialized in structure and function which are as given below:

1. **Lithocytes (Crystal cells):** Lithocytes are specialized epidermal cells containing crystals of **Calcium Carbonate (cystolith)**. It is present generally in multilayered epidermis. These crystals are of frequent occurrence in Apocynaceae, Acanthaceae, Moraceae *(Ficus)*, Cucurbitaceae and Utricaceae. Usually one systolith occurs in one lithocytes but in *Momordica sp.* (Cucurbitaceae), double cystoliths and even cystolith in groups are found in adjoining lithocytes attached to common walls.

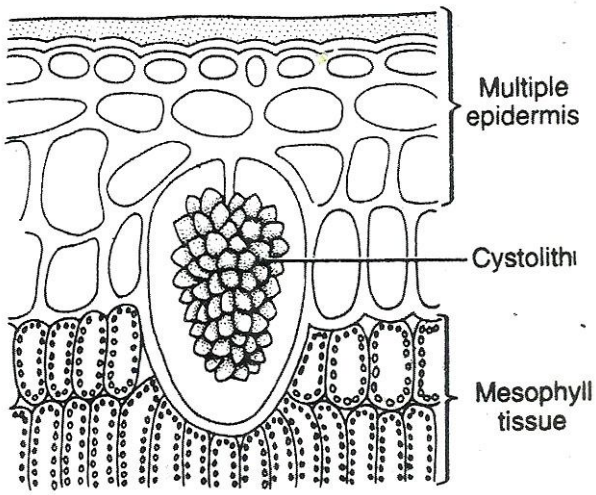


Fig. 4.2, Multilayered epidermis in *Ficus*

2. **Bulliform (Motor) cells:** These are the large water filled epidermal cells present in the epidermis of monocot leaves of xeric grasses. These cells are **turgidity sensitive (hygroscopic)** and brings about the rolling of leaves upward and inward in dry season to reduce the transpiration e.g. *Psamma, Poa, Ammophilla* etc.

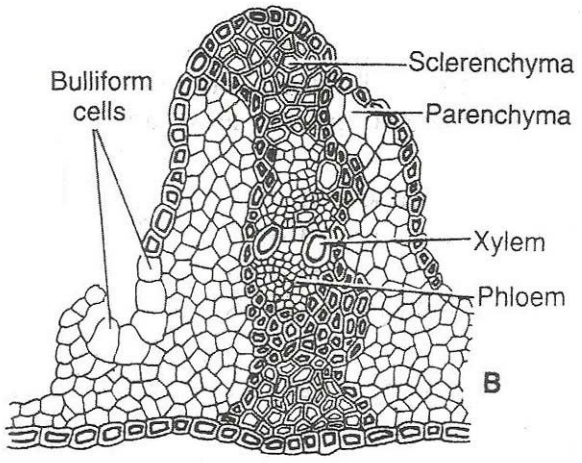


Fig. 4.3, T.S. of rolled leaf showing bulliform cells

3. **Epiblema and Trichoblast:** The root epidermis is commonly known as epiblema. It is also known as piliferous layer or rhizodermis. The cells which give rise to unicellular tubular extension (root hairs) are called **trichoblast.**

4. **Velamen:** Velamens are water absorbing tissue present in epidermis of aerial roots of some orchids and other epiphytes. The cells of velamen are compactly arranged and are dead. The main function of velamen is to absorb moisture from the atmosphere but it is also considered as a protective layer which checks evaporation of water from cortical cells.

5. **Silica and Cork cells:** In some of the Graminaceous plants e.g. *Hordeum*, there are two kind of cells above the vein; the long and the short; and the short cells are further of two types i.e. silica cells and cork cells. Both occur in pairs. The cork cells are suberized while the silica cells are filled with silica.

6. **Myrosin cells:** Some sac like cells is present in some cruciferous plant which contains enzyme myrosin.

Functions of epidermis:

1. Epidermis protects internal tissues against mechanical injury, fluctuations of temperature, attack of pathogens etc.
2. It checks water loss from internal soft organs.
3. It also helps in absorption, excretion, gaseous exchange and control of transpiration etc.

b. **Trichomes and emergences**: The cells of epidermis give rise two types of outgrowth i.e. emergences and trichomes.

1. **Emergences:** Emergences are those multi-cellulars out growth which comprise of epidermal and sub-epidermal tissues and thus they are more massive and complex in structure e.g. prickles in *Rosa* are emergences.
2. **Trichomes:** The outgrowth formed from epidermis only is called **trichomes.** Trichomes are small hairs on both vegetative and reproductive parts of the plants. Trichomes (epidermal hairs) may be unicellular or multi-cellular and found in all parts of the plants. Trichomes serve for checking excess loss of water and are also protective in nature. They can also alter heat loss from a plant, and act in storage and secretion of secondary metabolites. Trichomes have been regarded to be of significance in determining taxonomic status and phylogenetic relationship of angiosperms. Trichomes can be classified as hairs, scales and root hairs:

i. **Hairs** can be categorized as non-glandular or glandular. Non-glandular hairs are unicellular or multi-cellular. Staminal hairs of *Tradescantia* and seed coat hairs of cotton are the example of unicellular hairs. Multi-cellular hairs are found in *Helianthus*. Glandular hairs are secretary in function e.g. stinging hairs of *Urtica diooecia* and digestive glands of insectivorous plants *Drosera* are also the example of glandular hairs. Hairs present on all parts of plant and they may be of the following types:

1. Stellate hair – e.g. *Alyssum*
2. Grandular hair – e.g. *Solanum, Pelargonium*
3. Short glandular hair – e.g. *Lavandula*
4. Floccose hair – e.g. *Malva*
5. Stinging hair – e.g. *Cestus*

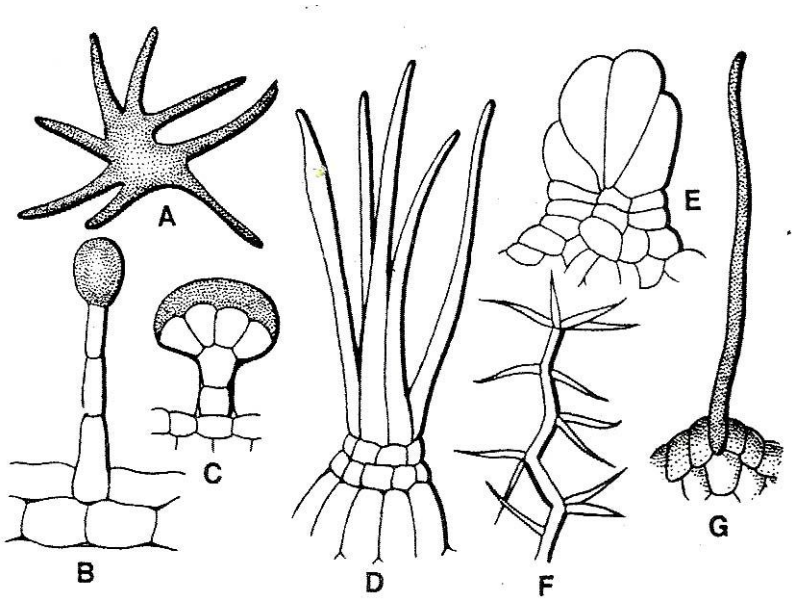


Fig. 4.4, Appendages of epidermis of leaves

A. Stellate hair of Alyssum, B. Glandular hair of Pelargonium, C. Short glandular hair of Lavandula, D. Floccose hair of Malva, E. Glandular hair of Solanum, F. Urticating hair of Verbascum, G. Stinging hair of Cestus

ii. **Scales** are the disc like plate of cells e.g. pitcher of *Nepenthes*.

iii. **Root hairs** are also trichomes that aid in water and mineral absorption. Only certain root epidermal cells termed as trichblast or piliferous cells can produce root hairs.

c. **Stomata:** Stomata are minute aperture in the epidermis of leaves and other aerial parts of the plant. Each aperture is bound by two specialized kidney shaped cells called guard cells. In guard cells chloroplasts are found abundantly. Guard cells are surrounded by a variable number of epidermal cells called subsidiary or accessory cells. The two guard cells, its aperture and accessory cells together constitute a stoma or stomatal apparatus. In some monocots like doob grass, *Zea mays* etc. guard cells are dumbbell shaped. Stomata in which guard cells and subsidiary cells are formed from the same mother cell are called **syndetochelic stomata.** Stomata in which guard cells and subsidiary cells are formed from two different mother cells are called **haplochelic type of stomata.**

Distribution of stomata: Although stomata occur in all aerial parts of the plants but they are found abundantly on leaves. Normally stomata occur on both the surfaces of leaves and such leaves are known as **amphistomatic** (e.g. potato). In floating leaves, stomata is present on upper epidermis only, such leaves are known as **epistomatic** e.g. *Nympheae*. Some time stomata are confined to lower epidermis of leaves and then the leaves are known as **hypostomatic** (e.g. *apple*). In submerged plants, the stomata is all together absent. Such leaves are known as **astomatic** e.g. *Potamogeton*.

Functions of stomata

1. They are avenues for gaseous exchange and hence they play important role in respiration and photosynthesis.
2. Most of the water absorbed by plant is transpired through stomata.

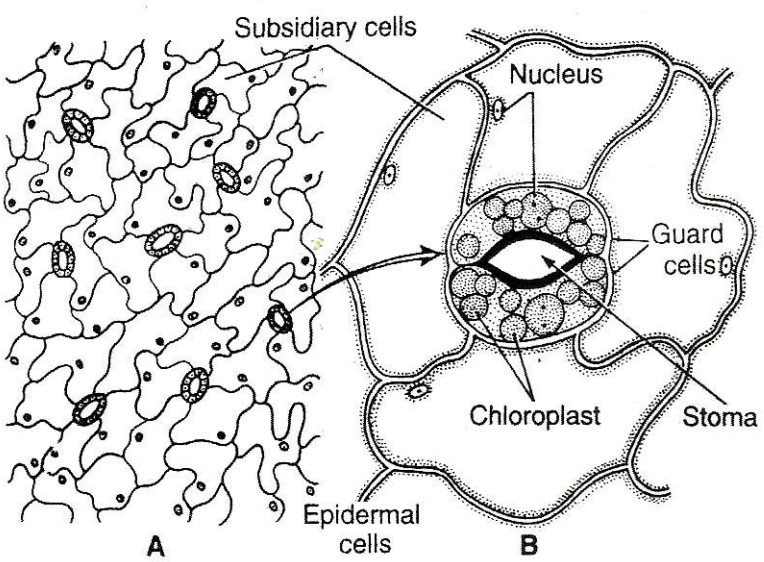


Fig. 4.6, A. Distribution of stomata on the dicot leaf,

B. Detailed structure of one stomata

d. **Periderm**: When plants increase in girth due to secondary growth, they slough off their epidermal tissues and replace them with periderm. The periderm is composed of cork cells (phellem) that have thick walls impregnated with suberin (a waxy substance which protects and waterproofs the surface of the cells). Cork cells are not very strong, and therefore are continually added to the

plant as it grows. Periderm may also contain un-suberized, thin-walled parenchyma cells called phelloderm. Water and gas exchange occurs through openings called lenticles. Although the function of lenticles is the same as the stomata, lenticles cannot control the size of their openings.

The corks found in wine bottles are cut from the bark of *Quercus suber*. In order to prevent wine bottle corks from leaking, they are cut at right angles to the lenticles

(B) Ground (Fundamental) tissue system: Ground tissue forms the main bulk of the plant body and extends from below epidermis to the centre (excluding vascular bundles). In roots & dicot stems, ground tissue system is of two types – extra-stelar ground tissue and intra-stelar ground tissue system. In monocot stems, as vascular bundles are scattered, hence no distinction between intra & extra-stelar ground tissue has been distinguished. Extra-stelar ground tissue also called **cortex** (lies between epidermis and the pericycle). Intra-stelar ground tissue present inner to vascular cylinder (includes pericycle and medullary rays & pith).

1. **Cortex:** There are three sub divisions of cortex from outer to inner side i.e. hypodermis, middle cortex and endodermis respectively.

a. **Hypodermis:** It is the outermost region of cortex which lies below epidermis. It consists of 3-5 or more layers. The hypodermis in stem and leaves of dicot is collenchymatous while it is sclerenchymatous in monocot & xerophytic plants. If ridges and grooves are present, collenchyma present in ridges and absent in grooves e.g. Cucurbitaceae. In stem and leaves, hypodermis gives mechanical support. In dicot stem, hypodermis performs photosynthesis and gives rise to **cork cambium.**

b. **Middle cortex:** Middle cortex (general cortex) lies between hypodermis & endodermis, which is multilayered, parenchymatous with prominent intercellular spaces. It helps in temporary storage of food materials and sometimes produces cork cambium or secondary meristem. In hydrophytes, generally cortex is arenchymatous.

c. **Endodermis:** Endodermis is inner most layer of cortex and is single layered. Cells are compactly arranged without any inter cellular spaces. In transverse section, cells of endodermis appear barrel shaped and are living. In endodermis of roots, casparian strips are present. Casparian strips discovered by **R. Caspary (1865)** and it is the thickening of **suberin** mostly and is present on the radial and inner tangential walls of endodermal cells. Casparian strips are the constant feature of roots of all plants but in stem, it is not very distinct. Just outside the protoxylem (opposite to protoxylem), some endodermal cells are thin walled which help in radial diffusion of water (from cortex to xylem) and are called **passage cells** (transfusion cells). Endodermis acts as **water dam** (water tight jacket) between vascular and non vascular region, maintain **root pressure,** and also act as **check post** that prevents leakage of nutrients from the vascular tissue and regulates inflow of water with mineral salts absorbed by roots through cortex directly into xylem elements. Endodermis also acts as **air dam** preventing the diffusion of air into the vessels. Endodermis store starch and hence also called as **starch sheath.**

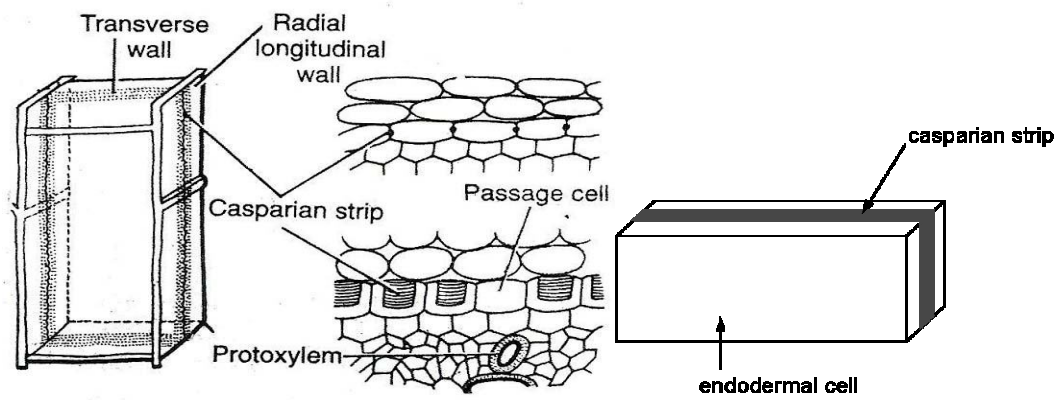


Fig. 4.7, Casparian strips and passage cells in the endodermis

2. **Pericycle:** It is present below endodermis, single layered or multilayered and constitute the outer boundary of primary vascular bundle. If pericycle consists of one type of cells (e.g. most of dicots), it is known as **homogenous pericycle**. It is known as **heterogenous pericycle**, if composed of two types of cells (parenchymatous as well as sclerenchymatous) in alternating bands (e.g. stems of Asteraceae). It is thick walled and multilayered in Cucurbitaceous stem**.** The pericycle of root is always parenchymatous. Pericycle is absent in the stems of monocots, hydrophytes and parasites (In *Smilax* roots, it is multilayered and sclerenchymatous). In dicot roots, it gives rise to part of cambial ring and also entire cork cambium originates from pericycle. It is the site of origin of lateral roots (endogenous in origin).

3. **Medulla (pith):** It is the intra-stelar region of the ground tissue, occupied the central position in dicot stem, dicot root and monocot roots. The cells are parenchymatous with intercellular spaces (occasionally sclerenchymatous). In most dicot roots, the pith is completely obliterated due to metaxylem elements**.** Parenchymatous or sclerenchymatous tissue present between the xylem and phloem of all roots is called **conjunctive tissue**. Parenchymatous extensions of pith in dicots stem in between the vascular bundles are called **medullary rays**.

(C) Vascular tissue system: Central column of axis (root and stem) is called stele which is made up of number of vascular bundles which constitute vascular tissue system. Each vascular bundle comprise of xylem, phloem and cambium (if present). First formed xylem is called proto-xylem and later formed as meta-xylem. On the basis of position of proto-xylem in vascular bundles, the primary xylem may be:

1. **Centrifugal:** Formation of xylem starts from the center i.e. Proto xylem near the centre and metaxylem towards periphery. This condition is known as **endarch condition** e.g. Stem of angiosperms & gymnosperms.

2. **Centripetal:** Formation of xylem starts from the periphery i.e. Proto xylem towards periphery (near pericycle) and metaxylem towards the centre. This condition is known as exarch condition e.g. Roots

3. **Centrifugal and centripetal**: Metaxylem is formed from both the side of the protoxylem (protoxylem surrounded by metaxylem), **mesarch condition** e.g. Rachis of Ferns and Rachis & leaflets of *Cycas*.

Types of vascular bundles

1. **Radial:** In radial vascular bundles, xylem and phloem lie on separate radii e.g. roots. In this the development of xylem is **centripetal** and the condition is **exarch.** (e**xception:** *Raddish, Carrot, Turnip, Sugarbeet*, in which vascular bundle is **conjoint collateral).**

2. **Conjoint:** In conjoint vascular bundles, the Xylem and phloem present on the same radius e.g. Dicot and monocot stem and leaves. Depending upon the mutual relationship of xylem and phloem, they are of two types:

a) **Collateral:** When xylem and phloem present on the same radius and xylem being internal and phloem external, such vascular bundles are called collateral vascular bundles. It may be open, if cambium is present between xylem and phloem (e.g. stems of gymnosperm and dicots angiosperms) or closed when cambium is absent (e.g. monocot stem). Development of xylem in this vascular bundle is centrifugal with protoxylem present in the centre (endarch condition).

b) **Bi-collateral:** When the two groups of phloem present on either sides of the xylem (i.e. xylem is sandwiched between outer & inner phloem). There are two strips of cambium, one on each side of the xylem. Development of xylem is centrifugal **(endarch**) e.g. stem of family Cucurbitaceae & some plants of family Apocynaceae and Solanaceae, Convolvulaceae.

3. **Concentric:** When xylem surrounds phloem completely or phloem surrounds xylem completely. Such vascular bundles are called concentric vascular bundles. It is always closed. It is of two types:

a) **Amphicribral (Hadrocentric):** When xylem is in the centre and surrounds by phloem on all sides. Such vascular bundles are called amphicribral vascular bundles e.g. Ferns and lower gymnosperms, *Lycopodium* etc.

b) **Amphivasal (Leptocentric):** When phloem is in the centre and surrounds by xylem on all sides. Such vascular bundles are called amphivasal vascular bundles e.g. *Dracaena, Yucca,* Pteridiophytes etc

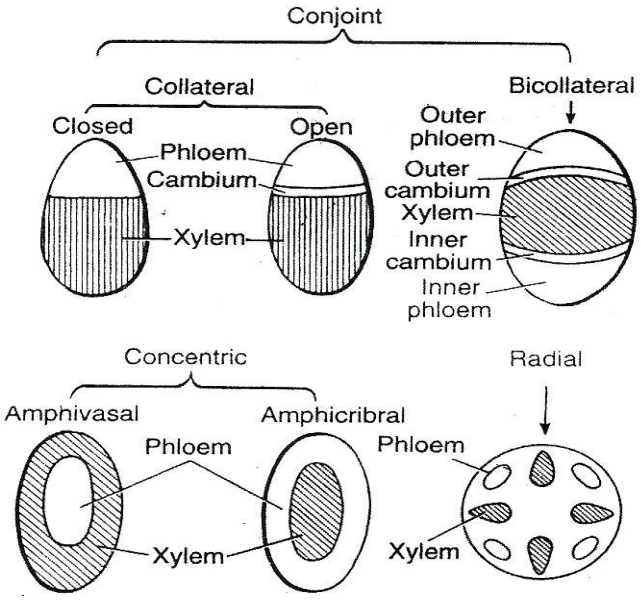


Fig. 4.8, Different types of vascular bundles

Table: 4.1, Difference between Vascular Bundles of Dicot and Monocot Stem

S. No.	Vascular bundles in dicot stem	Vascular bundles in monocot stem
1.	Uniform in size	Size variable
2.	Conjoint, collateral or bicollateral and open	Conjoint collateral and closed
3.	Phloem parenchyma are present	Phloem parenchyma are absent
4.	Bundle sheath is absent	Bundle sheath is present
5.	No water containing cavity is present	Water containing cavity is present
6.	Vascular bundles are arranged in a ring	Vascular bundles are scattered in ground tissues

Chapter-5

Secondary Growth

The growth in the length and formation of lateral appendages is called primary growth. It occurs due to activities of apical meristem and produces primary tissues which make the fundamental parts of the plants. Secondary growth may be defined as increase in girth or diameter due to addition of secondary permanent tissues formed with the help of primary & secondary lateral meristems i.e. vascular cambium and cork cambium in stelar and extra stelar regions respectively. Secondary vascular tissues are produced by vascular cambium while secondary ground tissues are formed by phellogen or cork cambium. Secondary growth is found in stems and roots of gymnosperms and dicots of angiosperms. No secondary growth found in monocot due to lack of vascular cambium. But exceptionally secondary growth found in some monocots such as *palm, Yucca, Dracaena, Smilax, Agave, Coconut etc.*

Secondary growth in dicot stems can be studied under the two heads: Secondary growth in stelar region and secondary growth in extrastelar (cortical) region.

Secondary growth in stelar region

Stelar secondary growth in dicot stems: The vascular bundles in dicot stem and stem of gymnosperms are conjoint, collateral, open and endarch and arranged in a ring. In stellar region, secondary growth occurs by the activities of vascular cambium. A strip of cambium that originates from the pro-cambium is located between the xylem and phloem of the vascular bundle. This cambium strip is called **intra-fascicular cambium**. At the onset of secondary growth additional strips of cambium are formed from the inter-fascicular parenchyma (pith rays) between the vascular bundles. These are known as **inter-fascicular cambium**. Both these cambia join and collectively constitute the complete ring of cambium which is now known as **vascular cambium** or **fascicular cambium** or **intra stelar cambium.**

Structure of vascular cambium: Two types of cells are found in a ring of this vascular cambium:

1. **Fusiform initial**: These cells are spindle shaped, vertically elongated forming axial system (longitudinal system) of secondary vascular tissue i.e. secondary xylem & secondary phloem (form vascular elements e.g. vessels, trachieds, sieve tubes etc.).
2. **Ray initials:** These cells are small, spherical, and horizontal in position forming radial system (give origin to rays cells i.e. elements of horizontal system of secondary vascular tissue (xylem rays in wards & phloem rays out wards – parenchymatous).

Activity of cambium and process of secondary growth: Activity of cambium depends on atmospheric condition. Cambium starts division in spring, remains continued through the summer and rainy season. Activity gradually slows down in autumn and in winter. A continuous periclinal division (tangential division) takes place in fusiform inintials. Of the two cells formed in this way, one daughter cell remains meristematic while other daughter cell behaves as xylem or phloem mother cell. If the daughter cell is on the outer side, it behaves as phloem mother cell and differentiates into secondary phloem and when formed on the inner side, it behaves as xylem mother cell which finally differentiates into secondary xylem. Generally vascular cambium forms more xylem than phloem because it is more active towards inner side. The ratio of xylem & phloem formed by cambium is generally 4:1 or 3:1. With the formation of secondary tissues, increase in girth or diameter occurs, which is thus called secondary growth. As the newer xylem is formed, the older is pushed in (towards the centre) and by the pressure of secondary xylem, all the primary tissue such as primary xylem, pith, old secondary xylem degenerate in the centre of the stem and because of this central part become woody. Similarly, the older phloem is pushed outward (towards periphery) after the formation of new phloem and due to presence of secondary phloem, primary phloem gets crushed. Thus epidermis and hypodermis cannot be seen during the secondary growth. Secondary Phloem is delicate structure, so, older cells are disintegrated due to outward push. But xylems are rigid structure, so, xylem cylinder remains present as a layer. Activity of cambium is going on continuously in plants throughout the life,

forming secondary xylem and secondary phloem. The ray initials of cambium cut plates of parenchymatous cells both outward and inward. These are secondary medullary rays (vascular rays) which extend horizontally from pith to secondary xylem and phloem.

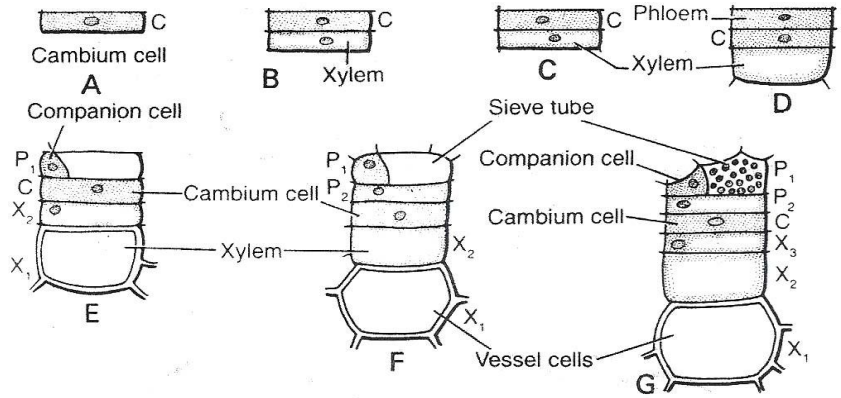


Fig. 5.1, Different stages in the development of secondary xylem and phloem by vascular cambium

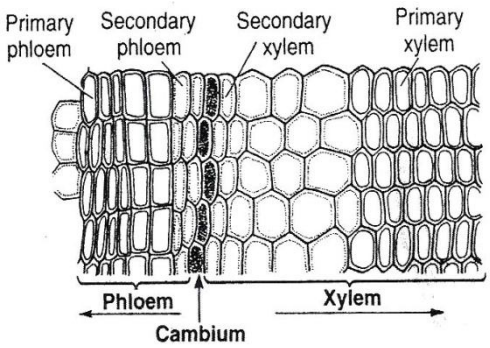


Fig. 5.2, Showing secondary xylem and phloem

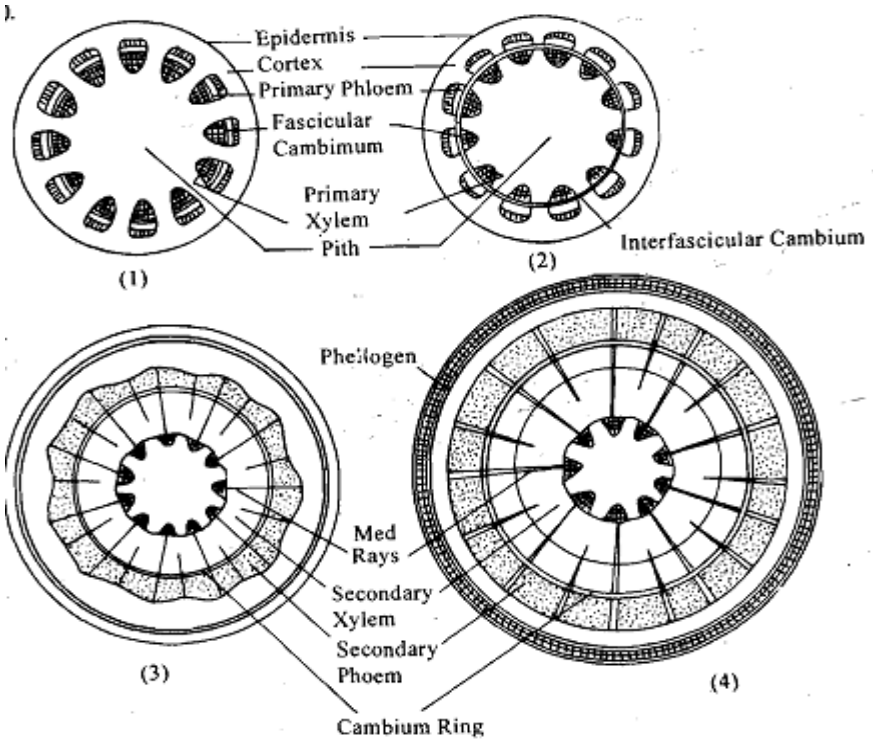


Fig. 5.3, Showing complete process of secondary growth in dicot stem

Secondary xylem (wood): The secondary xylem forms the bulk of vascular tissue in woody plants. The secondary xylem has two systems of tissues, the axial system and the radial system which show different orientation in the longitudinal axis of the plant. The axial system consists of vertical files of tracheary elements, fibers and wood parenchyma, whereas the radial system consists of rows of parenchymatous cells oriented at right angles to the longitudinal axis of the plant and forms vascular rays.

Xylem and phloem rays (vascular rays): At some places, the cambium does not form secondary xylem and secondary phloem but form parenchymatous cells instead of xylem and phloem. Thus these cells form continuous strips from secondary xylem to secondary phloem and are called secondary medullary rays. These rays are arranged radially. Primary and secondary medullary rays conduct food, minerals and water from centre to periphery.

Annual rings (growth rings) and spring and late wood: In woody plants that grow in temperate regions, the cambium shows marked variation in its activity in different seasons. There is seasonal alternation of periods of activity and quiescence. In spring, the activity of cambium is more and hence the wood elements are larger in size with wide lumen and more in number and called **spring wood or early wood.** In winter or autumn, the activity of cambium is less and the wood elements are smaller in size, with narrow lumen and less in quantity and called **autumn wood or late wood or winter wood.** It is more compact. The autumn and spring wood is formed in a ring. The ring of any type of wood is called **growth ring**. Thus two growth rings are formed during a year, a ring of autumn wood and a ring of spring wood. These two rings are collectively known as **annual ring**. Thus an annual ring consists of two growth rings. The number of annual rings formed in a tree gives the idea of the age of the tree.

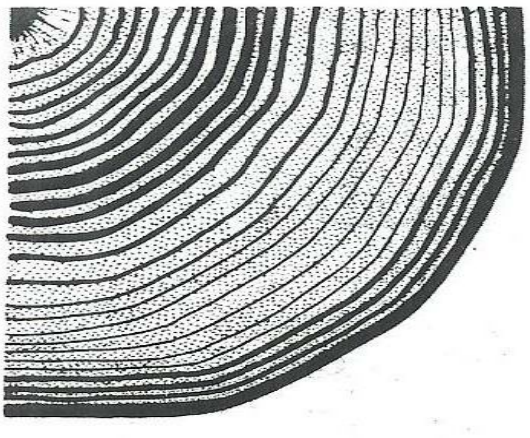


Fig. 5.4, showing annual rings in an old stem

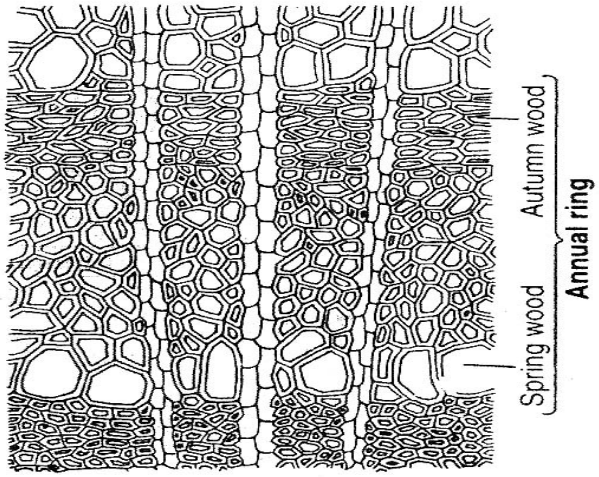


Fig.5.5, showing one annual ring

Dendrochronology: The branch of science in which the age of a plant is determined by counting annual rings is called **Dendrochronology.** The annual rings are counted from the base of the stem because basal part having maximum annual rings and upper part have less. Therefore, counting from the basal region can give the correct idea. A piece of wood is taken from the stem up to central region with the help of **increment borer instrument**. The annual ring counted from that piece and again inserted (fitted) into the same stem at the same place. Annual ring is formed more distinct in changeable seasons. A more distinct annual ring is formed in temperate plants. A distinct annual ring is not formed in tropical plants. A clear annual ring is not formed in India except Himalayan regions. Lesser distinct annual ring is formed in seashore regions because their climate remains the same throughout the year. More distinct annual ring is formed in deciduous plants as compared to evergreen plants. In roots, however, the annual rings are not usually well marked because the soil temperature remains almost uniform throughout the year.

Sap wood and Heart wood: As a result of continuous secondary growth of several years, the older parts of the stem have a part of its secondary xylem rendered non-functional. It is always the central portion of the xylem of which the vessels and trachieds become filled with waste material such as lignin, suberin, tannin, resin, gums etc due to degeneration of cells. Because of this, wood in the central region of the stem becomes dark coloured (black brown). It is called heart wood (Duramen). The peripheral or outer wood which looks light in colour are called sap wood (Alburnum). Development of Tyloses (tyloses are tracheal plugs, which plug the lumen of vessels) also makes the heart wood non-functional. The conduction of water now performed only by saps wood. With the passage of time and addition of new outer rings of secondary xylem, more ring of sap wood is changed into heart wood where as the sap wood remains about the same thickness. This is a gradual process during which the living cells of sap wood lose their protoplast, vessels are blocked by tyloses and there is impregnation of substances like gums, tannin, suberin etc. Conduction of water is not carried by heart wood because:

a) Cavities of trachieds and vessels progressively filled by waste materials.

b) Formation of tyloses in lumen through the pits in their wall

If the heart wood destroyed in any stem, then there will be no effect on plants. But if sap wood destroyed, the plant will die because conduction of water will blocked. Heart wood provides support. Waste material of heart wood are antiseptic in nature, resistant to bacteria and fungus, have power of repelling insects, so it is resistant to the termites. This heart wood is the best quality of wood. Study of wood is known as Xylotomy.

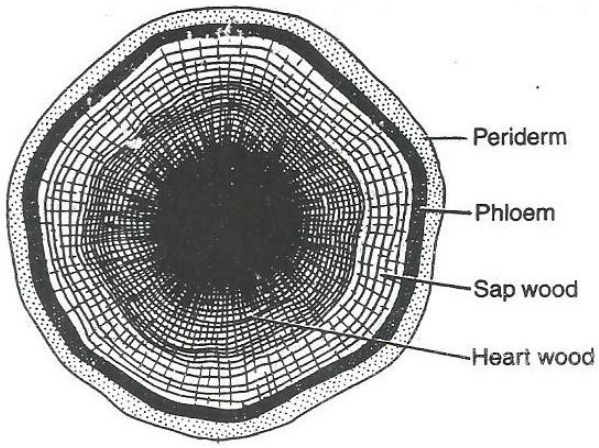


Fig. 5.6, Heart wood and sap wood in a T.S. of old dicot stem

Tyloses: In many plants, the walls of the xylem vessels produce a balloon like out growth into lumen of the vessels which is known as tyloses. It is in growth of neighboring parenchyma cells which block the lumen of the vessels and normally it develop in heart wood of angiosperms. It prevents rapid entrance of water, air and fungus by blocking the lumen of the vessels.

Tylosoides: In the wood of conifers, there is also found a closing of the cavity of resin canals by the enlargement of epithelial cells. These enlarged cells are commonly known as tylosoides.

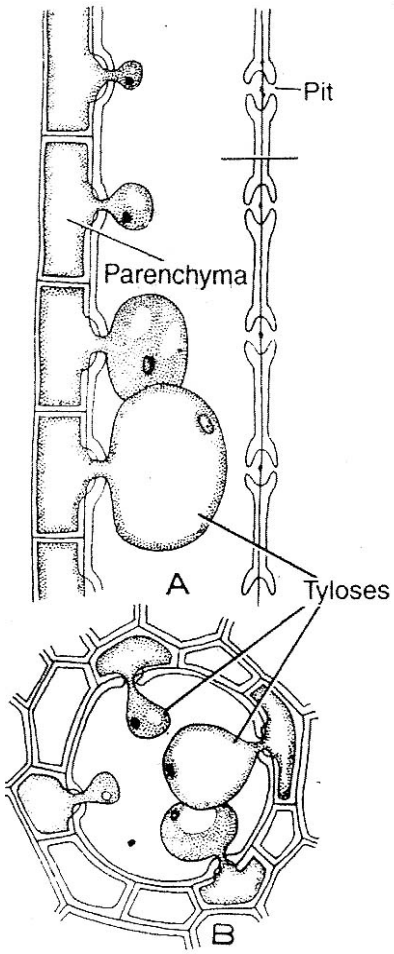


Fig. 5.7, Tyloses

A. L.S. and B. T.S.

Classification of wood On the basis of amount of parenchyma

1. **Manoxylic wood:** Manoxylic wood contains more living tissue of parenchyma and less amount of xylem. This wood is soft and loose e.g. *Cycas* etc.
2. **Pycnoxylic wood:** Pycnoxylic wood contains less amount of living tissue of parenchyma and more amount of xylem. Wood is harder. Such wood are found in most of the plants e.g. *Pinus, Mango, Acasia, Teak, Delbergia* etc.

Classification of wood on the basis of presence or absence of vessels:

1. **Non porous wood (soft wood): W**ood that lacks vessels is called non porous wood e.g. Gymnosperms because opening of vessels do not appear near cut end of them. (90-95% trachieds & 5-10% of ray cells). The most durable soft wood is that of *Cedrus deodara.*
2. **Porous wood (hard wood):** In mostly dicots, the vessels are widely present so, near the cut end of the stem opening of the vessels appear as pores and the wood is called porous wood. On the basis of arrangement of vessels, porous woods are grouped further into two types:

a) **Ring porous wood:** Vessels are arranged in the form of a ring in this wood. The vessels formed in the early part of the season are much of larger diameter than the later formed vessels. Thus rings of wide and narrow vessels occur. This type of wood said to be ring porous wood. Such wood conduct water more efficiently e.g. *Tillia, Tamarindus, Quercus, Morus* etc. (Temperate region)

b) **Diffused porous wood:** Asymmetrical distribution of vessels is found in this wood i.e. vessels are uniformly scattered in the secondary xylem e.g. *Neem, Jamun, Acer, Betula* etc. (Tropical regions). There is no appreciable difference in the size of early and late wood and they are nearly of the same size throughout the season.

Secondary growth in cortical region: A protective tissue usually replaces the ruptured epidermis due to the pressure of the increasing secondary vascular tissues. This protective tissue is said to be periderm. Preiderm formed by the activity of phellogen (cork cambium of secondary lateral meristem). Development of periderm is a common phenomenon in stems and roots of dicot and gymnosperms. Periderm also develops when a stem or root is wounded and is termed as wound periderm.
Structure of periderm: It is made up of three types of tissues:

1. Phellogen (cork cambium)
2. Phellem (cork layers) and
3. Phelloderm (secondary cortex)

1. **Phellogen (cork cambium):** It is secondary lateral meristem that may arise from the permanent living cells of sub-epidermal layers usually or even from the epidermis itself. Histologically, the phellogen is relatively simple and is composed of only one type of cells. Phellogen is also formed in a single layered ring. In transverse section, the cells appear almost rectangular and radially flattened. The cells of cork cambium show the following characteristics:

a. The cells are thin walled.

b. They have vacuolated protoplast.

c. The cells may contain chloroplast and tannin.

The cork cambium remains alive for one year. Each year, a new cambium is formed below previous cambium. This new cambium derived from secondary cortex or phelloderm.
Phellogen undergoes periclinal division. It forms some cells towards outside (epidermis) and some cells towards inside (cortex). If the outer cell functions as phellogen and remains meristematic, the inner cell becomes phelloderm layer (secondary cortex). In case inner one remains meristematic (phellogen), the outer ones transform into cork layer. Cork is formed in higher quantity and phelloderm in less quantity.

Position and origin of cark cambium: Regarding its origin and position, the phellogen may be categorized as:

a. In most of the stem, the first phellogen arises in sub-epidermal layer (hypodermis) e.g. *Prunus*.

b. Phellogen arise from epidermis e.g. *Pyrus, Nerium*.

c. Phellogen arise from cortex e.g. *Larix, Pinus, Bougainvillea*.

d. Phellogen arises from phloem e.g. *Vitis, Berberis.*

Note: Phellogen cells divide both in periclinal as well as anticlinal planes. But periclinal divisions are more. Periodic anticlinal divisions occurring in the phellogen cells to keep pace with the increasing circumference of the axis.

2. **Phellem (cork):** As a result of periclinal divisions in phellogen, the cells which cut off towards outer side mature into cork cells. They are compactly arranged and are polygonal and uniform in shape. They are living and have cellulosic walls in the beginning but at maturity, it become dead and is characterized by suberin deposition on their walls. Suberin is impervious to water. Phellem prevents the inner tissue from drying. Cork cells may also contain resinous and tanniferous material which gives brownish colour.
   Some time non-suberized cells also occur in cork cells which are known as phelloids. In *Betula*,

alternating layers of suberized and non-suberized cells are present and cork peels off like a sheet of paper.

3. **Phelloderm (secondary cortex):** Due to tangential (periclinal) division in phellogen, the cells which cut off towards inner side form phelloderm. The cells of the phelloderm are living cells with non-suberized cellulose walls, which have simple pits. Chloroplasts may also be present in the cells of phelloderm of some plants. They resemble cortical cells and often distinguishable from cortex by heir radial alignment with the phellogen and phellem.

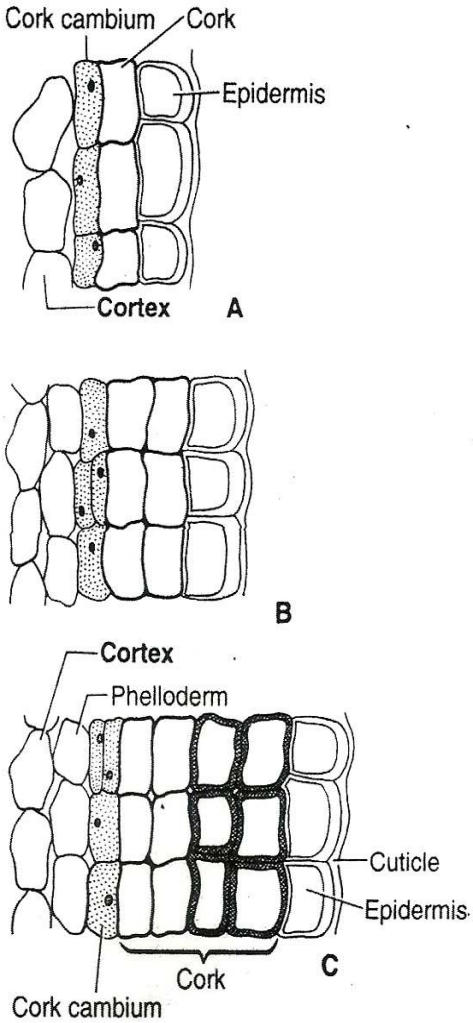


Fig. 5.8, Formation of periderm

Bark: Bark is a loose term and used to define all the tissues outside the vascular cambium**. (**Bark = periderm +cortex + pericycle + primary and secondary phloem.). The bark has two parts:

1. **Outer bark:** The entire tissues lie outside the cork cambium is called outer bark. Outer bark is dead. It is also known as rhytidome.
2. **Inner bark:** The region in between the cork cambium and vascular cambium is called inner bark. Its most part is living. The main region of the inner bark is the secondary phloem.

Thus bark constitutes both types of tissues–living and non-living (dead). A plant will die if we remove the complete bark of the plants because maximum loss of water occurs from this. There are two views about the bark which are as:

1. **Modern view:** All the tissues situated outside the vascular cambium are called bark. It is the composition of all the tissues outside the vascular cambium which includes periderm, primary cortex, pericycle, primary and secondary phloem. According to most scientists, all the above mentioned tissues are included in bark and all the dead tissues outside the cork cambium are included in cork. According to modern view, bark includes both living and dead tissues.
2. Old **View:** All the tissues outside the cork cambium are called bark. According to old views, bark only includes dead tissues.

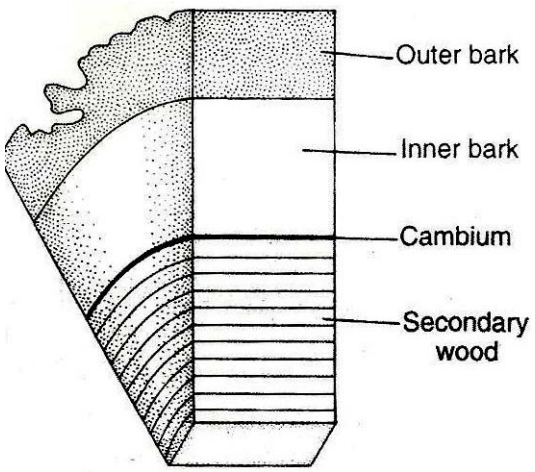


Fig. 5.9, Outer and Inner bark

Kinds of Bark: There are two kinds of bark:
Ring Bark: Here the ring of cork cambium is complete and that is why the bark produced in the form of a complete ring e.g. *Betula utilis* (Bhojpatra). In ancient times, it is used as writing material as paper. Ring bark also present in *Vitis, Clemitis* etc.
Scaly bark: Bark formed around stem in the form of pieces. When the ring of cork cambium is not completed, the scaly bark is formed. Highly obvious scaly bark is formed in *Psidium guava*, Neem, Mango, *Eucalyptus*, *Quercus* etc.
Uses of barks: Quinine is obtained from the bark of Cinchona tree (*Chinchona officinalis*). The bark of *Cinnamon* (dalchini) is used as flavoring materials. The bark of Juglans is used for cleaning and shining of teeth. The bark of *Acacia* is commercially used in tanning. The bark of *Betula utilis* used in ancient time as writing material.

Commercial cork: The phellem (cork) of *Quercus suber* (cork oak) is the source of commercial cork. In this plant, the first phellogen arises in the epidermis and persists indefinitely but when the tree is about 40 cm in circumference, at about the age of 20 years, the first formed periderm called virgin cork removed and now the new phellogen formed deeper in the cortex which produces cork rapidly. The cork is removed as sheet after 10 years when it is sufficiently thick and of commercial value and subsequent stripping is done after each 10 years up to 150 years of the age. In Cork Oak, which yield bottle cork, the cavities of cork cells (lumen) are filled with air which make the cork light in weight. Suberin also resists the oil. The air present in the cavities also gives thermal insulating qualities. Its light weight, resistance to pressure, thermal insulating qualities, imperviousness to liquids and resistance to acids and other chemicals make it commercially important.
Rhytidome: In most of the woody plants, due to continued growth of secondary nature, the first phellogen replaced by deep seated phellogen which excceds up to phloem in successive years. As phellogen arise in deeper region, it cut off cells of cork outside. All the living cells outside the phellogen do not get water and nutrients supply and become dead. These dead tissues formed outside the phellogen constitute rhytidome.
Lenticels: Most of the cells of phellem are dead, but at some places living cells are also found. Suberin is not deposited on this region. This place is called lenticels. It helps in breathing & transpiration. Wutz (1955) defined lenticels as a small portion of periderm where the capacity of phellogen is more

than elsewhere and the cork cells produced by it are loosely arranged with many intercellular spaces. These loosely arranged cells with many intercellular spaces are called lenticels. They are present in most of the woody plants undergoing secondary growth and help in gaseous exchange during night or when the stomata are closed.

Origin and structure of lenticels: Its formation initiates just below the stomata and its number corresponds to the number of stomata. Cells below stomata divide in different planes to form a mass of rounded cells. These cells grow bigger in size, lose their contents and become colourless. They form the first layer of complementary cells or filling tissue. Such cells also produced by phellogen beneath complementary cells instea of cork cells and so the number of complementary cells increase and pressure caused against epidermis and it ruptures. Lenticels are present in most of roots and stem undergoing secondary growth. The absence of lenticels can be attributed to annual peeling off of external layers of bark. The peeling off of the bark at regular intervals brings the inner tissue in contact with fresh air.

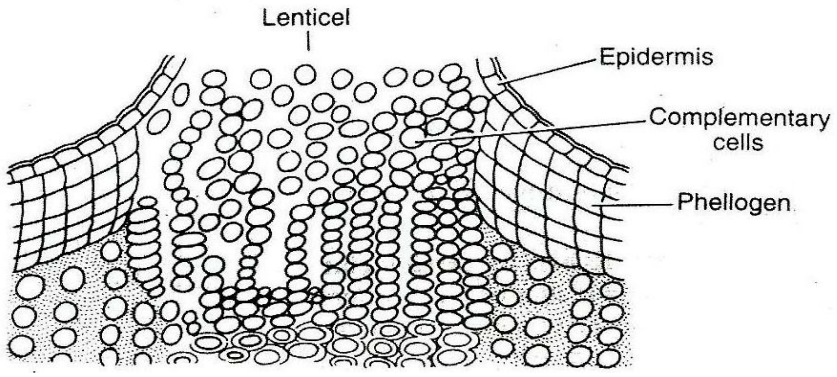


Fig. 5.10, Showing lenticel

Healing of wounds and wound cork: Wounds in plants are formed due to external injuries. Healing of wounds is important for the plant protection otherwise bacteria, fungi or other microbes may cause disease. Besides this, additional evaporation from the wound area may cause damage to the plants. In case of superficial wound, the exposed cells die and dry up. In case of deeper wounds, the uninjured cells below the wound become meristematic and produce a mass of undifferentiated parenchyma cells. This is known as **callus**. A wound cambium (inducible cambium) develops in peripheral layers of the callus which forms the wound cork towards the outside. The wound cork heals up the wound. Sometimes, the callus over grows the wound and forms characteristics knot on the stem. Wound cork may develop in all parts of the plants including fruits and leaves. Usually wound cork develops more easily on woody plants than the herbaceous plants. Successful development of wound periderm is important in horticultural practices when plant parts used for propagation are apt to be injured by handling or must be cut e.g. potato tubers, sweet potato roots etc.

Abscission: The leaves of most pteridophytes are either falls after degeneration or destroyed on the plants. The leaves in gymnosperms and woody dicots are separate through the abscission before their death. Middle lamella is dissolved in abscission layer and primary walls also dissolved partially or completely. The place where leaf separated is called **leaf scar**. The living cells present in leaf scar are responsible to form cork cambium and cork is formed towards the outside and so that ultimately the relation of the leaf detached from the plant. This is termed as abscission. Abscission layer is composed of parenchymatous cells.

Secondary growth in dicot root: During the secondary growth in dicot root, the following events take place:

Initiation and activity of vascular cambium: The vascular bundle in dicot roots are radial and diarch to hexarch. Xylem and phloem are arranged on separate radius in a ring. During secondary growth, parenchymatous cells of conjunctive tissue lying below the phloem patches become meristematic and form crescent shaped strips of cambium. As the activity of these cambium strips proceeds, the cells of pericycle external to each xylem strand also become meristematic and small strips of cambium are

also formed outside the xylem strands. The first formed strips of cambium inner to phloem strands join with the newly formed strips of cambium outside the xylem strands. Thus a complete but wavy ring of cambium is formed which runs outside the xylem and inside the phloem strands. The first formed cambium strips start functioning earlier than the later formed cambium. Due to formation of secondary xylem opposite the primary phloem strands the cambium in these regions is pushed outwards. Now the wavy cambium ring becomes circular in outline. This cambium ring produces secondary phloem on the outer side of secondary xylem on the inner side.

Initiation and activity of cork cambium: A ring of cork cambium is differentiated in pericyle or cortex. It produces phellem (cork) towards outer side and phelloderm (secondary cortex) towards inner side. The first formed phellogen may persist for a considerable period of time. When it becomes inactive, it is replaced by successively more deep seated phellogens. The three tissues (phellem, phellogen and phelloderm) constitute the periderm. The cells of phellogen undergo divisions both on the outer as well as on the inner side. The derivatives formed on the outside are rectangular and compactly arranged, become suberized, tannin is deposited in the cell interiors. Cork being suberized is impermeable to water. Being dead and tannin containing air filled tissues, cork protects the root interior from pathogens, changes in temperature and mechanical injury. A few layered secondary cortex is formed on the inner side of the cork cambium called phelloderm. Phelloderm, phellogen and phellem are collectively called periderm.

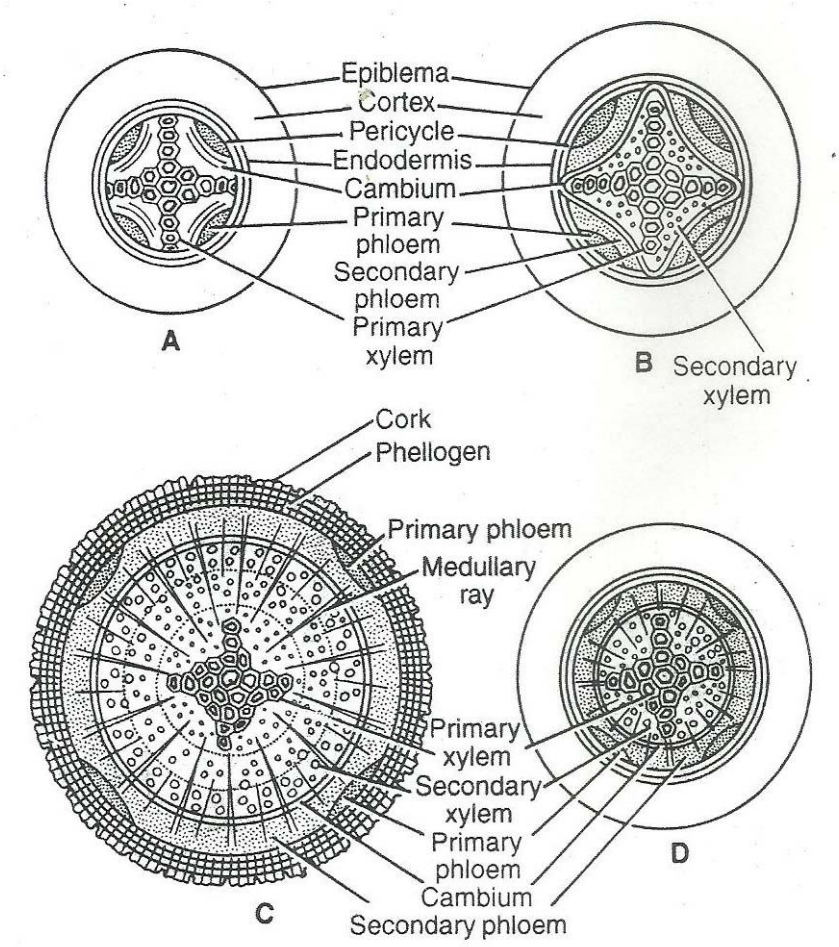


Fig. 5.11, Different stages in secondary growth of dicot root

Secondary Growth in Monocotyledons: In some members of the plants such as *Dracaena, Yucca, Agave, Aloe* etc. vascular cambium formed from the outer region of ground tissue. Parenchyma is formed towards outer side from vascular cambium and vascular bundles are formed towards inner side. In some plants the girth of the stem increased without cambium e.g. *Palms, Musa* etc. The apical

meristem of these plants is special type which is known as Primary Thickening Meristem. This apical meristem is responsible for growth in both length and girth of the plants.

Anomalous growth: The structures which differ from the normal structure are known as anomalous. With the results of the combination of unusual structure, some complex structures are formed which are known as anomalies. Such anomalies are very common in angiosperms. These anomalies are as:

1. **Absence of vessels in xylem:** In the xylem of angiosperms, normally vessels are present but there are some primitive angiosperms which lack vessels in their xylem e.g. *Trochodendron* of family trochodendraceae, *Tetracentron* of family tetracentraceae, *Pseudowintera* of family winteraceae. In some aquatic plants e.g. *Ceratophyllum, Hydrilla* etc. vessels are also absent in the xylem.
2. **Presence of scattered vascular bundles:** Generally in dicot stems, the vascular bundles are arranged in a ring but in some plants, vascular bundles are found scattered e.g. *Thalictrum, Nymphea, Papaver orientale, Anemone, Podophyllum* etc.
3. **Presence of meduallry vascular bundles:** In many dicots, Vascular bundles in pith region are found in addition of normal ring of vascular bundle. These are known as medullary vascular bundles. They may be arranged in a ring or may be scattered e.g. *Amaranthus, Boerahvia, Chenopodium, Mirabilis, Achyranthus, Bougainvillia, Raphanus, Pepromia* etc.
4. **Presence of cortical vascualr bundles:** In some dicots, vascular bundles also present in cortex in addition to normal vascular bundles e.g. *Casurina, Nyctanthes, Lathyrus* etc.
5. **Intraxylary phloem:** This is also known as internal phloem. Phloem on innermost radius called as internal or intraxylary phloem or medullary phloem. The origin of these phloem is primary in nature e.g. *Calotropis, Caspicum, Leptadaenia* etc.
6. Some vascular bundle exclusively formed from only xylem (no phloem element) in addition to normal collateral vascular bundles e.g. *Paeonia*
7. Some vascular bundles exclusively formed from phloem (no xylem elements) in addition to normal vascular bundles e.g. *Cuscuta*

Anomalous secondary growth in Dicotyledons: Many dicot plants show deviation from the normal secondary growth described earlier. This type of growth is called anomalous secondary growth. Due to this type of secondary growth many dicot stems have unusual secondary structures. These unusual secondary structures are due to the following reasons:

a) **Abnormal position of cambium:** In many dicots, position of cambium is abnormal and stem become unusual in shape e.g. in *Thinouia scandens*, while stem is young, in the cambium several fold are develop which later separate and also separate steles develop from these.

b) **Abnormal behaviour of normal cambium:** In the stems of *Clematis* and *Vitis*, only parenchyma is produced by the interfascicular cambium. As a result, primary vascular bundles remain discrete throughout the secondary growth. In *Aristolochia, Bauhinia, Tinospora* etc. the complete cambium ring is active but the fascicular cambial strip forms secondary vascular tissue where as the interfascicular strip gives rise to parenchyma cells. As a result, the xylem becomes fissured due to the development of broad parenchymatous (medullary) rays. In *Bignonia*, a common garden climber, the cambium ring shows normal activity in the beginning but after some times, the cambium forms internally lesser amount of secondary xylem and on outer side greater amount of phloem at four diagonal places. It results in formation of four deep wedges of phloem projecting into the secondary xylem which gives the appearance of phloem islands **(internal phloem).** In *Leptadenia* and *Salvadora*, **phloem islands** occur in secondary xylem. At some places, though cambium is normal but behaves abnormally for some times and cuts secondary phloem inside instead of secondary xylem and again after some time becomes normal producing secondary xylem inside the cambium and secondary phloem outside as usual. In this way phloem appears in between secondary xylem as island which is known as **internal phloem** or **interxylary phloem**. Interxylary phloem is secondary in nature.

c) **Formation of accessory (successive) cambium ring:** Formation of successive rings of cambium may lead anomalous secondary growth. In some plants e.g. *Boerhaavia diffusa, Bougainvillea* and *Mirabilis*, the normal ring of cambium stops functioning after producing a secondary vascular cylinder of secondary phloem and secondary xylem. New cambial ring is formed outside i.e. centrifugally in the pericycle or in the cortex. Like this, successive cambial rings appear and produce alternating concentric rings of secondary vascular tissue consisting of secondary phloem and xylem. These **internal phloems** are secondary in origin and occur in between the secondary xylem.

Extra-stelar cambium: Extra-stelar cambium develops in pericycle e.g. *Amaranthus, Achyranthes, Chenopodium* etc. In *Amaranthus*, the extrastelar cambium cuts off cells only towards inner side. At certain places, these cells develop into secondary vascular bundles and at certain places, it forms conjuctive tissues. The vascular bundles remain embedded in conjuctive tissues

Abnormal structure in monocot stems

a) In *Triticum, Secale, Hordeum, Avena, Oryzae* etc. (Family Graminae), vascular bundles situated in ring.

b) In *Aristolochia* fluted vascular cylinder is formed.
In *Bauhinia* strap like stem is formed.

c) In *Urtica* Islands of parenchyma is formed

d) Intra-xylary cork is formed in *Achiellea, Epilobium* etc.

Chapter-6

Internal Structure of Root, Stem and Leaf

Internal Structure of Root: The transverse section of the root shows the following arrangements of tissues from the periphery to the centre.

1. **Rhizodermis (epiblema):** It is the outermost layer made up of single layer of parenchymatous cells without intercellular spaces which is also known as **piliferous layer**. Stomata and cuticle are absent. Outer wall of the epiblema forms unicellular tubular prolongation called root hairs. These hairs help in absorption of water from the soil. Epiblema is usually uniseriate, but in aerial roots of some orchids and epiphytic plants, multiseriate epidermis called **velamen** is present.
2. **Cortex:** Cortex consists of oval or rounded parenchymatous cells with plenty of intercellular spaces. These cells may store food reserves. In some cases (e.g. *Iris*) the epiblema soon dies off and a few outer layer of cortex become cutinized and form the exodermis. The cells of exodermis have suberized cell walls. The cortical cells store starch but in aerial roots of *Tinospora*, it contains chloroplasts and thus become green and performs photosynthesis.
3. **Endodermis:** It is made up of single layer of barrel shaped parenchymatous cells without intercellular spaces which completely surrounds the stele. The radial and the inner tangential walls of endodermal cells are thickened with suberin. These thickenings are known as **casparian strips**. Casparian strips are absent in those endodermal cells which are located opposite to protoxylem elements and are known as **passage cells (transfusion tissues)** which allow radial diffusion of water.
4. **Stele:** All the tissues present inside endodermis comprise the stele which include pericycle, vascular tissues and pith (if present).

a. **Pericycle:** Pericycle is generally a single layer of parenchymatous cells found inner to the endodermis. Lateral roots originate from the pericycle. Pericycle is single layered as in *Sunflower* or multilayered thick as in *Mulberry*.

b. **Vascular system:** Vascular tissues are always in a ring and radially arranged i.e. xylem and phloem is situated at different radii. Xylem shows exarch condition. The number of vascular bundles in dicot roots are generally 2-6 (diarch to hexarch) and in monocot roots, it usually ranges from 6 to 20 (polyarch condition) but the polyarch condition is also found in *Ficus* (Banyan tree) which is a dicot. Metaxylem vessels are generally polygonal in shape.

c. **Pith:** Pith is very short or absent and consists of parenchymatous cells. In many dicots, due to the development of metaxylem vessels in the centre, pith is obliterated.

5. **Conjunctive tissues:** The parenchyma cells lying in between xylem and phloem bundles forms the conjunctive tissues. Vascular cambium is formed from conjunctive tissues during secondary growth.

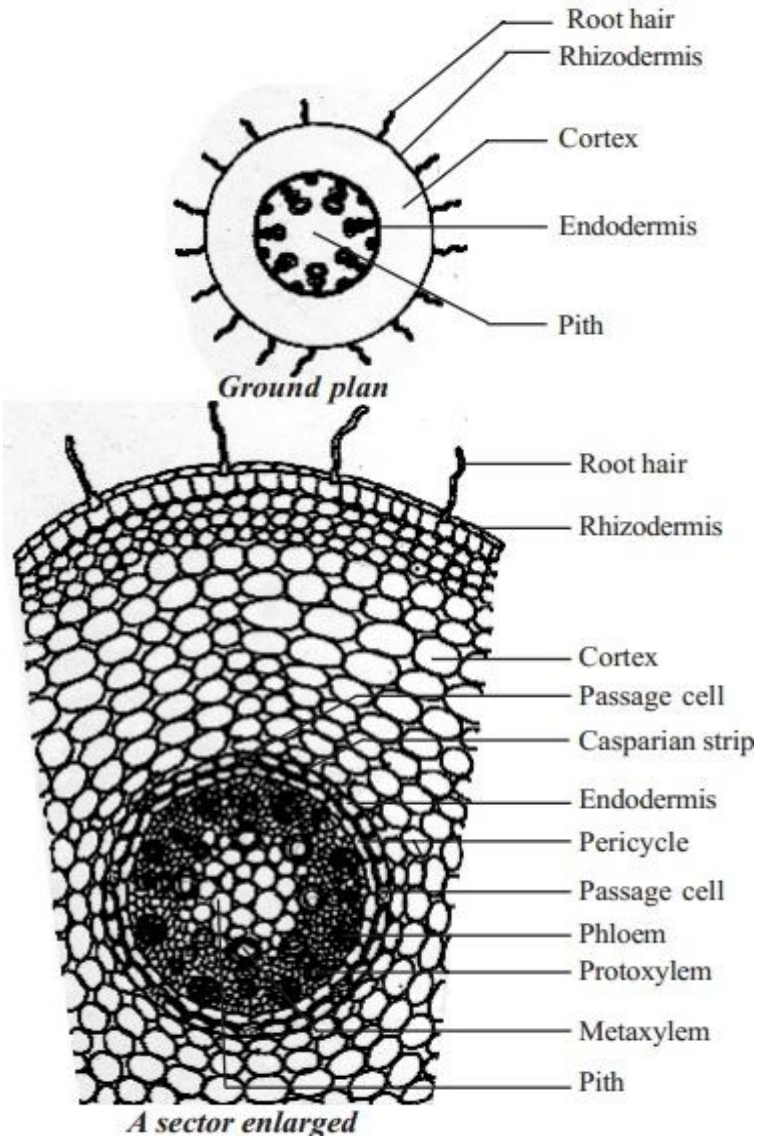


Fig. 6.1, T.S. of monocot (Maize) root

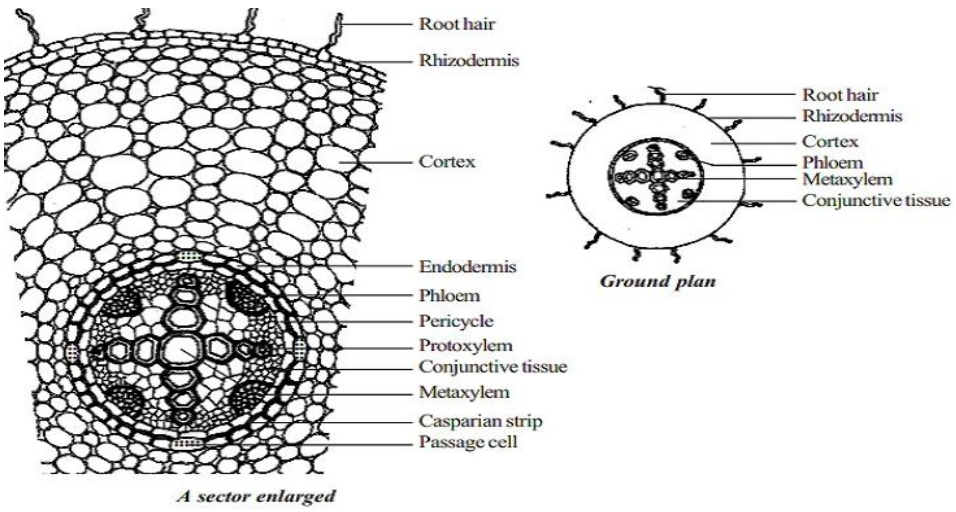


Fig. 6.2, T.S. of dicot (Bean root (Pith is absent in this case)

Heteroarchy in Roots: In some plants, roots of different arches are present in the same plant. Such roots are called **Heteroarchic roots** and phenomenon is called **Heteroarchy**. **Wardlaw (1928)** first time reported phenomenon of Heteroarchy in *Nymphaea chilensis*. Here the following conditions are found at different stages of development e.g.

1. Young roots show tetrarch condition
2. Slightly mature roots show 7 arch conditions.
3. Still more mature roots show 12 – 14 arch condition.
4. Most mature roots show 16 – 18 arch condition.

Heteroarchy is also present in *Eryngium* (umbilleferae), *Enhydra* (Compositae).

Contractile Roots: Roots of certain dicots (herbaceous) and monocots show wrinkling on their surface at maturity e.g. *Medicago, Daucus, Trifolium, Crinum, Allium* etc.

Table: 6.1, Anatomical Difference Between Monocot and Dicot Roots

S. No.	Monocot roots	Dicot roots
1.	Pericycle gives rise to lateral roots only	Pericycle give rise to lateral roots, phellogen and parts of vascular cambium
2.	Xylem is usually polyarch	Xylem is usually tetrarch
3.	Pith is usually well developed and large	Pith is usually absent
4.	Metaxylem vessels are generally circular in crosss section	Metaxylem vessels are generally polygonal in cross section
5.	Conjuctive tissue is sclerenchymatous in *Maize*	Conjuctive tissue is usually paraenchymatous
6.	There is no secondary growth	Secondary growth is generally present

Internal Structure of Stem: The transverse section of the young stem shows the following arrangement of tissues from the periphery to the centre.

1. **Epidermis:** Epidermis is the outer most layer of stem. It is made up of single layer of cells which are cutinized and lack chloroplasts. Multicellular hairs (trichomes) and stomata are found on epidermis.
2. **Cortex:** Cortex can be divided into three regions:

a. **Hypodermis:** It is present just below the epidermis. It is thick and multilayered. This layer is composed of collenchyma and their cells contain chloroplast. So, the hypodermis is green and photosynthetic. In cylindrical stem, it forms a continuous ring but in angular stem e.g. *Cucurbita,* it occurs in patches.

b. **General cortex:** It lies inner to hypodermis and consists of few layers of thin walled parenchymatous cells. Intercellular spaces are abundant. Chloroplasts may present in the outer layer of cells. Latex tubes, resin ducts, crystals of calcium oxalate and reservoir of waste products are frequently present in the cortex.

c. **Endodermis:** It is single layered and inner most layer of the cortex. The cells of this layer are barrel shaped arranged compactly without intercellular spaces. Due to abundant starch grains in these cells, this layer is also known as **starch sheath**. The starch sheath of stem is homologous to endodermis, but is not so specialized. It is better to call it as **endodermoid.**

3. **Stele:** It consists of pericycle, vascular bundles and pith.

a. **Pericycle:** Pericycle occurs between the endodermis and vascular bundles. It is multilayered and may be parenchymatous (e.g. most of the dicots) or sclerenchymatous (e.g. *Cucurbita)* or both parenchymatous and sclerenchymatous in patches (e.g. Asteraceae).

b. **Vascular bundles:** In dicot stem, vascular bundles are arranged in a ring around the pith. Each vascular bundle is conjoint, collateral, open and endarch.

c. **Medullary rays:** A few layers of big polygonal cells lying in between two vascular bundles are the medullary rays. These store water and food material and also function in lateral conduction.

d. **Pith:** The large central portion called pith composed of parenchyma cells with intercellular spaces. The extension of pith between vascular bundles is called as pith ray or medullary rays. Function of the pith is storage of food.

Table: 6.2, Anatomical Difference between Dicot and Monocot Stem

S. No.	Dicot stem	Monocot stem
1.	Hypodermis is collenchymatous	Hypodermis is sclerenchymatous
2.	Ground tissue is differentiated into cortex and pith.	Ground tissue is not differentiated but it is a continuous mass of parenchyma
3.	Vascular bundles are arranged in ring and are generally uniform in size (eustele)	Vascular bundles are scattered and are of various size, usually large towards the center (atactostele)
4.	Vascular bundles are conjoint, collateral, open and endarch	Vascular bundles are conjoint, collateral, closed and endarch
5.	Vascular bundles are not surrounded by sclerenchymatous sheath	Vascular bundles are surrounded by sclerenchymatous sheath (bundle sheath)
6.	Pericycle is present	Pericycle is absent
7.	Medullary rays are present	Medullary rays are absent
8.	Phloem parenchyma is present	Phloem parenchyma is absent
9.	Pith present	Pith absent
10.	Secondary growth occurs	Secondary growth generally absent

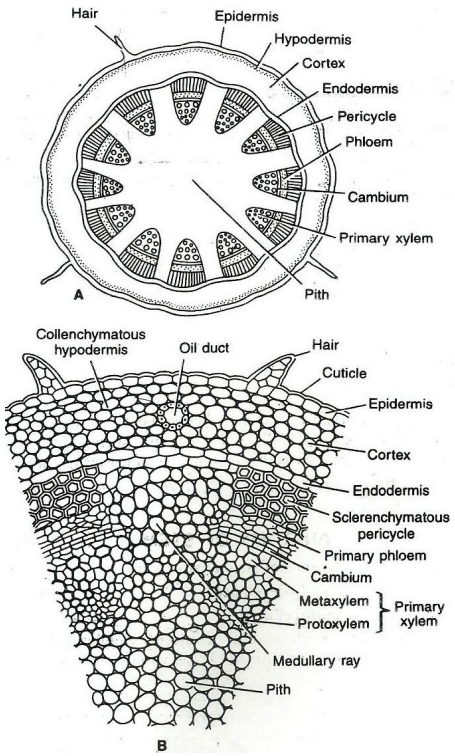


Fig. 6.3, Dicot stem (sunflower)

A. T.S. diagrammatic B. Portion enlarged

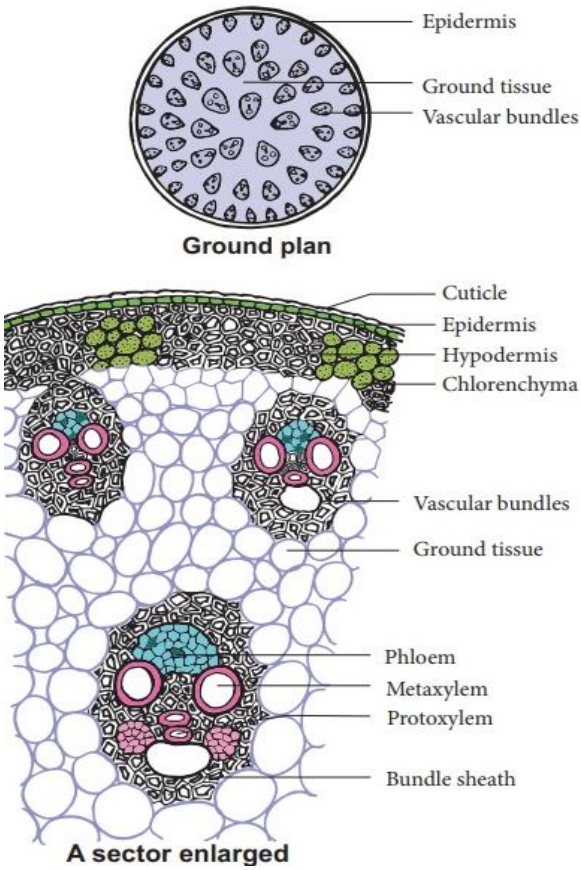


Fig. 6.4, T.S. of monocot stem (Maize)

Internal Structure of Leaf: Like root and stem, the leaf also consists of three types of tissue systems, the dermal tissue system consisting of upper and lower epidermis, the ground tissue system consisting of mesophyll cells (the main photosysnthetic tissue) and the vascular system comprising of veins. Common leaves are **bifacial** which are of two types i.e. dorsiventral and isobilateral. **Unifacial leaves** are found in garlic and onion in which there no differentiation is found between upper and lower surface. In **albascent leaves**, the palisade parenchyma restricted to only half part of the leaf, so, half leaf appears greener and other half appear less green.

A transverse section through the mid rib region of a typical dorsiventral leaf reveals the following structure:

1. **Epidermis:** Both upper and lower epidermis is usually made up of a single layer of cells that are closely packed. The cuticle on the upper epidermis is thicker than that of lower epidermis. Multiple epidermis is found in some plants e.g. *Ficus, Nerium* etc. Some epidermal cells in *Ficus l*eaves contain **cystolith** and called **lithocytes.** Stomata are more in number on the lower epidermis than on the upper epidermis. In monocots, the leaves are isobilateral and possess almost equal number of stomata on the both the surfaces. The main function of the epidermis is to give protection to mesophyll. The cuticle helps to check transpiration. Stomata are used for transpiration and gas exchange. In some plants (e.g. *Nerium*) stomata are present in sunken cavities called stomatal crypts. In some xerophytic grasses, few cells in the upper epidermis are enlarged to form motor cells called bulliform cells which help in rolling and unrolling of leaves. When the bulliform cells in the leaves have absorbed water and are turgid, the leaf surface is exposed. When they are flaccid due to water stress, they make the leaves curl inwards to minimize water loss.

2. **Mesophyll:** The entire tissue between the upper and lower epidermis is called the mesophyll which is differentiated into palisade and spongy parenchyma. In monocots, the mesophyll cells are not differentiated into palisade and spongy parenchyma.

a. **Palisade parenchyma:** Below the upper epidermis, vertically elongated cylindrical cells in one or more layers without intercellular spaces form palisade parenchyma. Palisade parenchyma cells contain more chloroplasts than the spongy parenchyma cells. The function of palisade parenchyma is photosynthesis.

b. **Spongy parenchyma:** Below palisade parenchyma towards lower epidermis, irregularly shaped, loosely arranged cells with numerous air spaces form spongy parenchyma. Spongy cells facilitate the exchange of gases with the help of air spaces. The air space that is found next to the stoma is called **respiratory cavity** or **sub-stomatal cavity.**

3. **Vascular tissues (in the veins):** Vascular bundles are conjoint, collateral, endarch and closed. Xylem is present towards the upper epidermis, while the phloem towards the lower epidermis. Vascular bundles are surrounded by a compact layer of parenchymatous cells called bundle sheath or border parenchyma. Protoxylem vessels are present towards the upper epidermis. Phloem consists of sieve tubes, companion cells and phloem parenchyma. Phloem fibres are absent. Xylem consists of vessels and xylem parenchyma. Tracheids and xylem fibres are absent. Xylem is mesarch. The vascular bundle near the mid rib is largest while these become smaller towards the margins. In monocots, the vascular bundles are similar to dicot leaf but all vascular bundles are similar in size. In monocots, the bundle sheath of the midrib vein is connected to the upper and lower epidermal layers by sclerenchyma cells representing bundle sheath extensions or hypodermal sclerenchyma. Bundle sheath cells also contain chloroplast but without grana. Bundle sheath is **chlorenchymatous in xerophytic** (C4 plants), remaining plants have sclerenchymatous bundle sheath. In most grasses & cereals, leaves show Kranz anatomy where dimorphic condition of chloroplast is found.

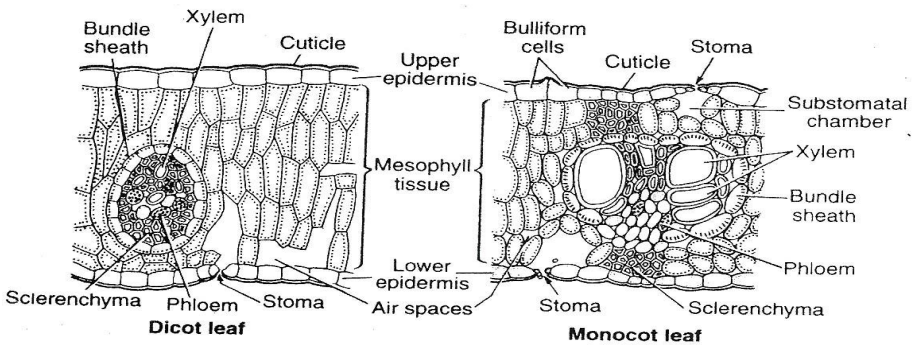


Fig. 6.5, Showing the comparative account of dicot and monocot leaves

Table: 6.3, Anatomical Difference between Dicot and Monocot Leaves

S. No.	Characters	Dicot leaf	Monocot leaf
1.	Nature of orientation	Dorsiventral	Isobilateral
2.	Stomata	Mostly on lower epidermis	Equally distributed on both the surfaces of leaves
3.	Motor cells	Absent	Present in upper epidermis
4.	Mesophyll cells	Differentiated into palisade and spongy parenchyma	Undifferentiated
5.	Veins	Irregularly scattered	Parallely arranged
6.	Xylem	Many prtoxylem and metaxylem vessels in each bundle	Two protoxylem and two metaxylem vessels in each bundle
7.	Bundle sheath	Made up of collenchyma	Made up of sclerenchyma

Chapter- 7

Introduction

It is one of the fundamental tenets in biology that all life comes only from pre-existing life. Reproduction is one of the important characteristics of all living beings. In angiosperm plants, reproduction occurs by two methods namely vegetative and sexual reproduction. The plants of angiosperm like other groups of the plants, has two separated phases in the life cycle, the sporophyte and gametophytic phases which alternate with each other. This is known as alternation of generation. Hofmeister (1849) used the term alternation of generation. C. Wolf is Father of Plant Embryology and Prof. Pancham Maheshawari is regarded as **"Father of Indian Plant Embryology"**. A book **"An Introduction to Embryology of Angiosperm"** was written by Prof. Maheshawari.

1. **Sporophytic phase:** It is diploid (2n) phase. In angiosperms, the main plant body belongs to this phase. It is dominant stage and it is developed from diploid zygote. Reproductive organs (flowers) developed on this plant.
2. **Gametophytic phase:** It is haploid (n) phase. It is developed from haploid microspores and megaspores which are products of meiotic divisions of diploid microspore and megaspore mother cells respectively. Consequently the microspore and megaspores germinate and form the male and female gametophytes respectively. The male gametophyte gives rise to male gamete and female gametophyte forms the female gamete (egg cell). A diploid zygote is formed by the fusion of these gametes. The zygote undergoes repeated mitotic divisions and later become an embryo in the seed.

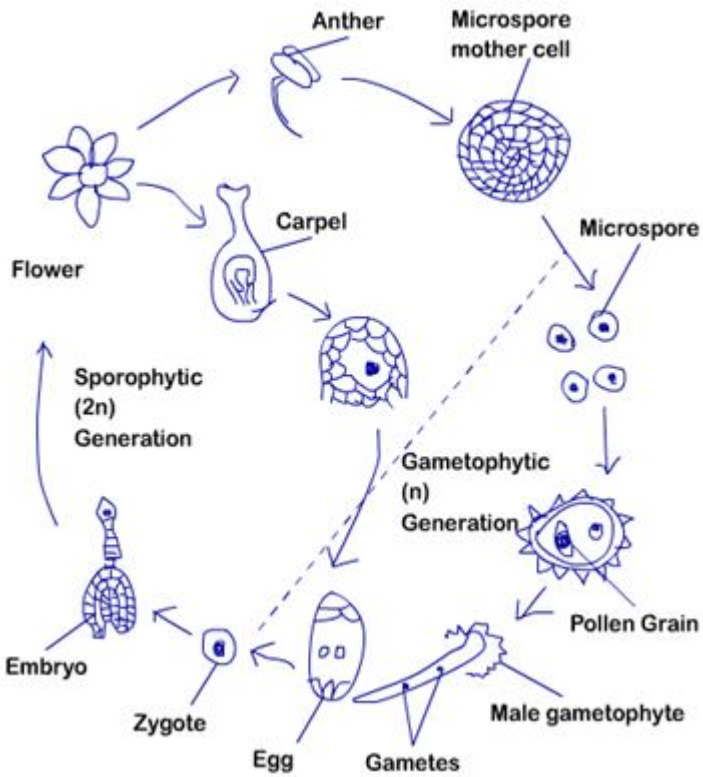


Fig. 7.1, Life cycle of an angiosperm

Homologies of flower parts:

Stamen	-	Microsporophyll
Anther (Pollen sac)	-	Microsporangium

Pollen grain	-	Microspore
Pollen with pollen tube	-	Male gametophyte
Carpel	-	Mega-sporophyll
Ovule	-	Mega-sporangium
Embryo sac	-	Female gametophyte
Egg	-	Female gamete

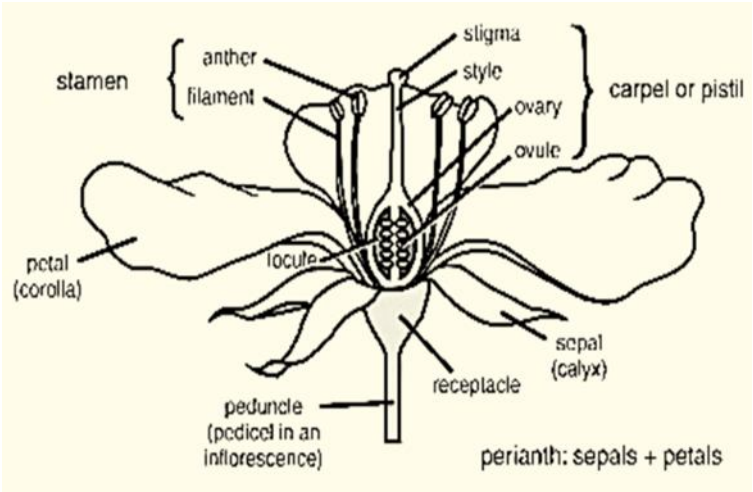


Fig. 7.2, L.S. of a typical flower

Sexual reproduction: Sexual reproduction occurs with help of flower. It has two essential floral organs known as reproductive organs e.g. Androecium and gynoecium. Sexual reproduction occurs by the formation and fusion of compatible pairs of gametes called male and female gamete. The whole process of sexual reproduction can be divided into the following steps

1. Pollen grain formation
2. Embryo sac formation
3. Pollination
4. Fertilization

Chapter-8

MALE REPRODUCTIVE ORGANS

Androecium, Stamen, Microsporangium and Pollen Grains: Androecium is male reproductive organ which consists of one or many stamens (microsporophyll). Stamen is the unit of androecium. N. Grew (1682) recognized that stamens are the male organ of flower. Stamen consists of two parts–a long slender stalk called filament (non-reproductive part of stamen) and a terminal structure called anther (reproductive part of stamen).

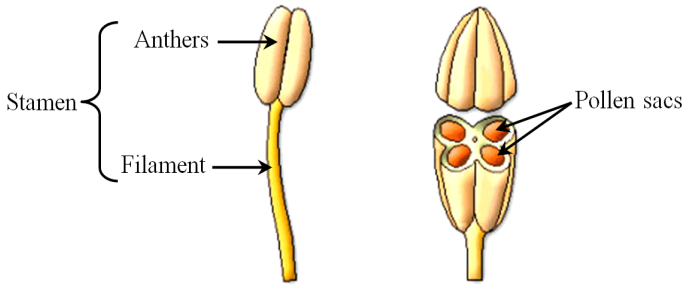


Fig. 8.1 Structure of stamen and section of anther showing pollen sacs

Anther

Anthers is reproductive part of stamens which is generally bi-lobed (each lobe is bithecus and each theca consists of two microsporangia); hence generally anthers are tetra-sporangiate. The development of anther is eusporangiate type (anther develops from a group of cells). Microsporangia later become pollen sacs producing pollen grains. Pollen grains represent male gametophytes. Pollen grains on development form two male gametes and one vegetative cell. First male gamete fuses with egg and forms a diploid zygote while second male gamete fuses with diploid secondary nucleus to form a triploid primary endosperm mother cell. Vegetative cell used up in formation of pollen tube during pollen germination on the stigma.

Initially a young anther is a homogenous mass of meristematic cells which is surrounded by single layered epidermis. On maturity it becomes two lobed. Each lobe contains two microsporangia. In each sporangium, a hypodermal cell become prominent with large nucleus and dense cytoplasm and behaves like archesporial cells. These cells divide by periclinal division (parallel to the outer wall of the epidermal cells) forming outer primary parietal cells and towards inner side a layer of primary sporogenous cells. By several anticlinal and periclinal division, primary parietal cells form 2–5 layered anther wall. Primary sporogenous cells develop into microspores.

Note: In Malvaceae family, there is a single lobe in anther (which is known as monothecus anther) with only one sporangium in each lobe and hence the anther is bisporangiate. In *Arceuthobium*, each anther contains only one microsporangium (monosporangiate anther).

Structure of mature anther: Each anther is bilobed. Each lobe consists of two pollen chambers (microspornagium). The two microsporangia are separated by a strip of sterile tissue called connective. Each microsporangium of an anther on maturity consists of sporogenous tissues covered by an anther wall.

Anther wall: Wall of a mature anther consists of epidermis, endothecium, middle layers and tapetum.

1. **Epidermis:** Outer most layer of anther wall is single layered epidermis which is useful for protection.
2. **Endothecium:** Endothecium is radially elongated which develop fibrous thickening of α-cellulose on their radial and inner walls which is hygroscopic in nature and help in dehiscence of anthers. The cells of endothecium opposite the partition between two microsporangia are thin

walled and constitute the stomium or line of dehiscenec through which pollens are discharged. (Fibrous thickening absent in anther of cleistogamous flowers).

3. **Middle layer:** Cells of middle layers are 1-5 layered and generally ephemeral in nature which store reserve food and degenerate before the pollen mother cells undergo meiosis and provide nourishment to pollen mother cells.

4. **Tapetum:** Tapetum cells are polyploids, multinucleated and coenocytic due to endomitosis and endopolyploidy**.** It is the inner most layer of anther wall and is single layered. Tapetum provides nutrition to developing microspores. Tapetum secretes ubisch bodies (orbicules) which get covered with **sporopollenin** and so increase the thickness of exine of microspores. Tapetum is of two types: (a) Amoeboid and (b) Secretary/ glandual layer

a) **Amoeboid or plasmodial or invasive type of tapetum**: In this, the inner and radial walls of tapetum break down at early stage and these cells are free in microsporangia or pollen chambers for providing nutrition to developing microsopres. It is of primitive type and found in *Arum, Lily, Typha, Alisma, Tradescantia, Butomus, Helianthus etc.*

b) **Glandular layers** (Secretary or parietal) type of tapetum: This type of tapetum is secretary in nature which remains intact throughout the microspore development. It is most common type of tapetum and perform the following functions as given below:

1. Secrete **callase** enzyme which dissolves **callose** (callose held together pollen grains in the form of tetrad).

2. It contains many small spherical bodies of only few micrometers in diameter called **Ubisch bodies or spheroids** (lipidic in nature). (The tapetal cells prepare pro-ubisch bodies (lipidic in nature) and gets coated with sporopollenin and become Ubisch bodies). Due to destruction of tapetal cells, the ubisch bodies come to lie in microsporangium and involve in the formation of exine.

3. It secretes oily and viscous secretion deposited on the pollen grains and form pollen kits. Pollen kits are the outer oily layer of many insect pollinated species and consist of lipids and carotenoids. (Pollen kit helps in insect pollination and protects pollens against harmful UV-rays).

Note: Under certain condition, the tapetum may haustorial and engulfs the developing microspore instead of providing nutrition. This may cause the male sterility.

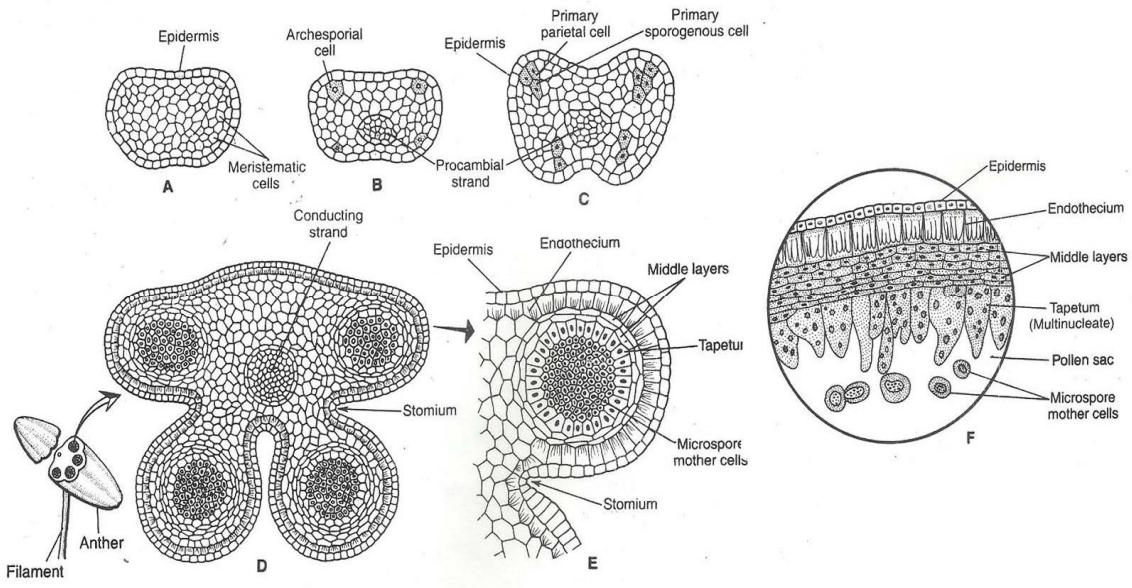


Fig. 8.2 Different stage of the development of anther

A. T.S. young anther, B. Differentiated archesporial cells, C. Differentiated primary parietal cells and primary microsporogenous cells, D. T.S. of young anther showing four microspornagia and vascular bundle, E. Detailed structure of one microsporangium, F. A portion of microsporangium showing details

Microsporogenesis: The primary sporogenous cells divide mitotically and produce sporogenous tissues which later behave as microspore mother cells or pollen mother cells. The microspore mother cells undergo meiosis and produce haploid microspore. Microspores remain in the form of tetrads. The formation of haploid microspores (pollen grains) from diploid microspore mother cells by meiotic division is called microsporogenesis. This process occurs in anther. There are two types of microsporogenesis:

1. **Successive type:** It is the common among monocots. In this, wall formation occurs after first as well as second meiotic division and thus an isobilateral pollen tetrad is formed.
2. **Simultaneous type:** It is common among dicots in which wall formed only once i.e. after completion of meiosis.

Note: Successive type of cytokinesis is considered to be more advanced as compared to simultaneous type.

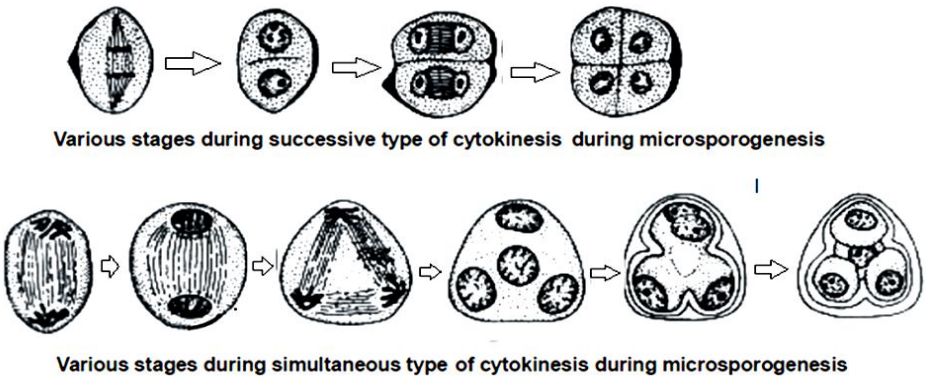


Fig. 8.3, Microsporogenesis

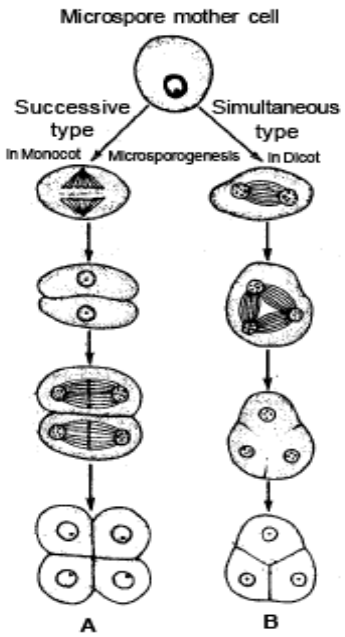


Fig. 8.4 Microsporogenesis

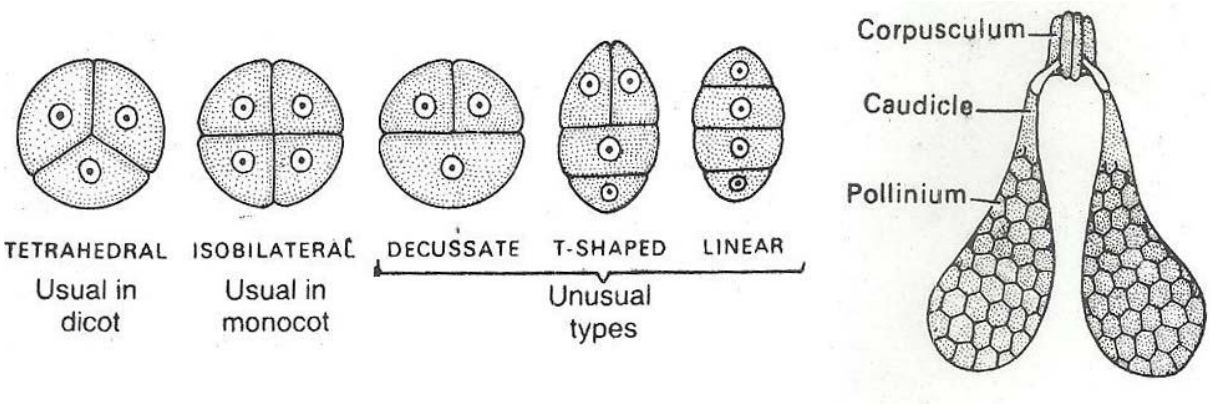
A. Successive type in monocotyledonous plants

B. Simultaneous type in dicotyledonous plants

Types of pollen tetrads: Microspores generally arranged in a tetrahedral or isobilateral fashion but there is some exception. In total, there are five types of pollen tetrads which are as:

1. **Tetrahedral tetrad**: It is most common type in dicot.
2. **Isobilateral tetrad:** It is most common type in monocots.
3. **Linear tetrad:** e.g. *Halophia* (Family – hydrocharitaceae)
4. **T-Shaped tetrad** e.g. *Aristolochia* and *Butomopsis*
5. **Decussate tetrad** (three microspore in one phase and one is in back) e.g. *Magnolia, Atriplex, Crocus* etc. (3, 4 and 5 types of tetrads are not common)

Note: In *Aristolochia elegans*, all five types of tetrads are found.



A **B**

Fig. 8.5, A. Different types of micropsore tetrads, B. Pairs of pollinia in Calotropis, making translator apparatus

Pollen grains: Mature pollen grain represents male gametophytes. Pollen grains are generally spherical measuring about 25-50 µm in diameter. It has a two layered wall, outer layer called exine and inner layer called intine. The term exine and intine was proposed by Fritsch (1837). Exine further differentiated into two layers i.e. an outer ektexine (sexine) and an inner endexine (nexine). Ektexine further differentiated into outer tectum, middle baculum and inner foot layer. In this way tectum is the outer most layer of exine. Tectum provides a characteristic sculpturing or designs over the surface of pollen grains. Exine is made up of sporopollenin which is the most resistant organic material of plant kingdom. It is resistant to high temperature, acids and alkali. Because of sporopollenin pollen grains are well preserved as fossils. Sporopollenin derived from oxidative polymerization of carotenoids. Intine is a thin and continuous layer made up of cellulose and pectin. The exine has one or more weak places known as germ pores through which intine comes out in the form of wall of pollen tube. Mature pollen grain consists of two cells, the vegetative cell and generative cell. Vegetative cell is bigger with abundant food reserve and an irregular shaped nucleus. Generative cell is small floats in the cytoplasm of vegetative cell. It is spindle shaped with dense cytoplasm and a nucleus.

Pollen grains are significant in causing allergies and respiratory disorders like asthma, bronchitis etc. e.g. *Parthenium* or carrot. Pollen grains are rich in nutrients. Pollen products are available in the market as pollen tablets and syrups. Pollen consumption has been claimed to increase the performance of athletes and horses. The branch of science which deals with study of the characteristics of pollen grains is called palynology.

Pollen viability: For effective fertilization to occur pollen should reach at stigma before viability is lost. The time period for which pollen grains have the capacity to produce male gametes for effective

fertilization to occur is the pollen viability. It is variable in different plants e.g. 30 minutes in rice and wheat. In others, the periods of viability is long, even months in some members of family rosaceae, leguminosae and solanaceae. It is however, depends upon environmental conditions of temperature and humidity. It is possible to store pollens for years in liquid nitrogen at –196 degree celsius in pollen for later use in plant breeding programme.

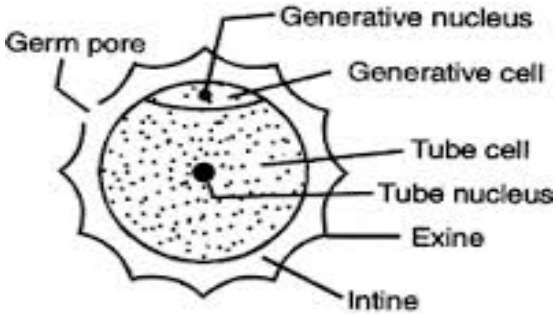


Fig. 8.6, Section of a mature pollen grain at two celled stage

Abnormal features present in development of pollens:

1. **Polyspory:** Some time more than four spores are formed from one spore mother cell. This phenomenon is known as polyspory e.g. In *Cuscuta reflexa***,** 11 pollen grains are formed from single pollen mother cells.
2. Normally spores separate from tetrad, but in *Hydrilla, Drimys, Anona, Juncus, Typha, Elodea, Drosera* and in the members of family mimosaceae e.g. *Albizzia, Acacia, Mimosa* etc.; pollen grains remain in tetrad and form compound pollen grains.
3. In some cases pollen grains of an anther aggregate and form a mass of pollen grains called pollinia (Massulae) e.g. *Calotropis, Neottia (Orchid).* In ascelpiadaceae and orchidaceae family, the pollinia of adjacent anther of different stamens are attached to a sticky disc called corpusculum by means of a stalk called caudicles and the whole structure is called as translator apparatus.
4. In some plants e.g. *Mimosa* (Mimosaceae), an intermediate condition is present i.e. 8-64 pollen are present in one group.
5. **Nemec phenomenon:** Nemec phenomenon is an interesting feature which is the formation of pollen embryo sac in *Hyacinthus orientalis*, in which nucleus of pollen grains goes under mitotic division and form eight nucleate embryo sac like structure. It was reported by Nemec in 1898.

Dehiscence of anthers: It is of the following types:

1. **Longitudinal slit dehiscence** e.g. *Datura, Gossypium, China-rose* etc. it is of common type.
2. **Transverse dehiscence** e.g. *Ocimum sanctum* (Labiatae)
3. **Porous dehiscence** e.g. *Solanum*, by apical pores
4. **Valvular dehiscence** e.g. *Barberis*

Development of Male gametophyte and Micro-gametogenesis: Development of male gametophyte is in-situ i.e. begins inside the microsporangium. The haploid pollen grains represent the first stage of male gametophyte. The nucleus of pollen undergoes mitotic division, and produce vegetative nucleus (tube nucleus or siphonogenic nucleus) and generative nucleus. The generative nucleus gets surrounded by cytoplasm to become generative cell and vegetative nucleus remains as such. Now at this 2-celled stage, pollination occur (pollen shed from microsporangium), further development of pollen occurs at stigma. (But in *Beta vulgaris, Portulaca, Hordeum* etc., pollen grains are shed at 3-celled stage). At stigma, after pollination, the pollen grains absorb water and other secretion secreted by stigma and swell. The exine ruptures and intine comes out through germ pore in the form of a tube which is known as pollen tube. The tube nucleus migrates to the tip of pollen tube. The generative

cells divide mitotically to form two male gametes, a small and a bit larger. Formation two male gametes from the generative cell is microgametogenesis. Pollen tube is nothing but the in growth of intine and secretes pectinase and other hydrolytic enzyme to create a passage for it in the style if later is solid. The pollen with pollen tube (having three nuclei) is fully developed male gametophyte in angiosperms. Amici (1824) discovered pollen tube and role of pollen in fertilization in *Portulaca oleracea* (pollen burst on stigma to form pollen tube).

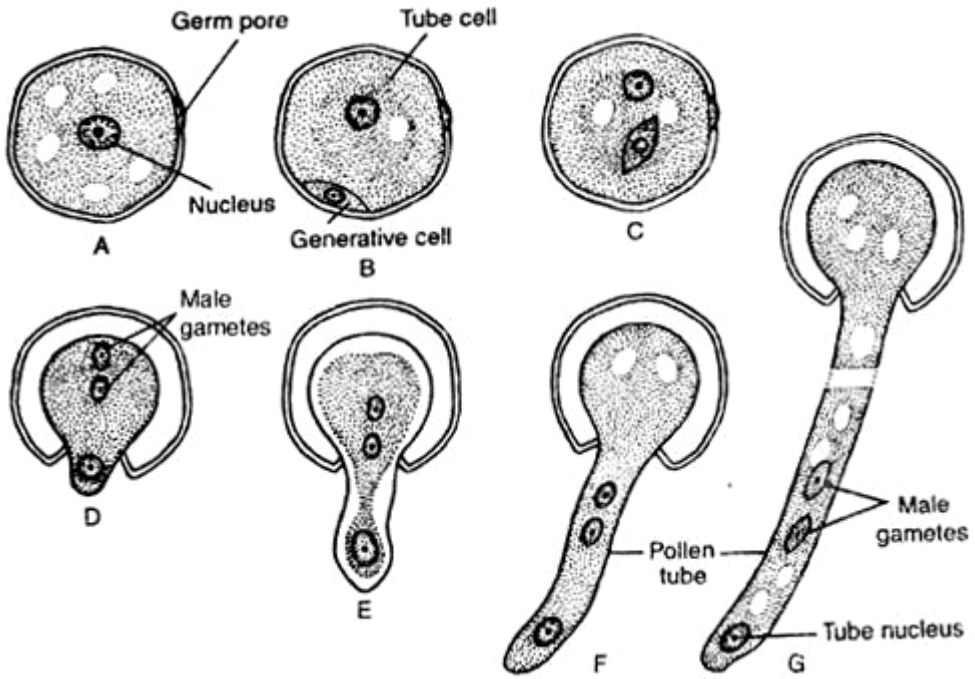


Fig.8.7, Germination of pollen germination and development of male gametophyte in angiosperm

Chapter-9

FEMALE REPRODUCTIVE ORGAN

Gynoecium (pistil), carpel, ovary, ovule and embryo sac: Rudolph Camerarious (1694) discovered Pollination and sexual reproduction in plants and recognized pistil as female sex organs. Gynoecium or pistil is the female reproductive organ, consists of one or many carpels. Carpel is the unit of gynoecium. Each carpel consists of an ovary, a style (pollen tube receptive part) and a stigma (pollen receptive organ). Both stigma and style are sterile while ovary is the fertile part of carpel. Ovary bears one or more ovules (megaporangium) which contains embryo-sac (female gametophyte). Generally embryo-sac is 8 nucleated and 7 celled structures which contain egg apparatus (one egg + two synergids), secondary nucleus or polar nuclei (n + n) and 3 antipodal cells.

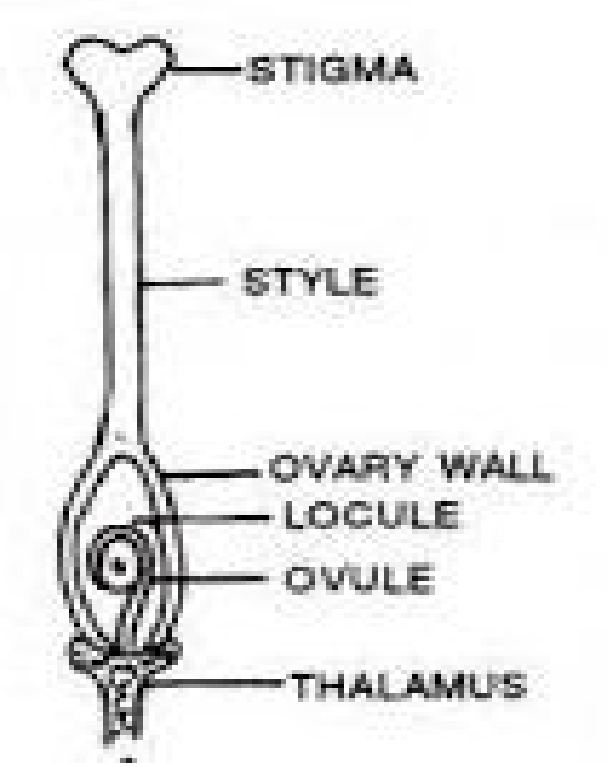


Fig. 9.1, Structure of a typical carpel

Ovule: Technically, ovule is megasporangium. The various parts of an ovule are as described below:

1. **Funicle:** It is a stalk-like structure which represents the point of attachment of the ovule to the placenta of the ovary.
2. **Hilum:** It is the point where the body of the ovule is attached to the funicles.
3. **Integuments**: They are the outer layers surrounding the ovule that provide protection to the developing embryo.
4. **Micropyle:** It is a narrow pore formed by the projection of integuments. It marks the point where the pollen tube enters the ovule at the time of fertilization.
5. **Nucellus:** It is a mass of the parenchymatous tissue surrounded by the integuments from the outside. The nucellus provides nutrition to the developing embryo. This is known as megasporangium proper. Mature ovule develops embryo sac inside nucellus from the single megaspore.
6. **Chalazal:** It is the basal swollen part of the nucellus from where the integuments originate.

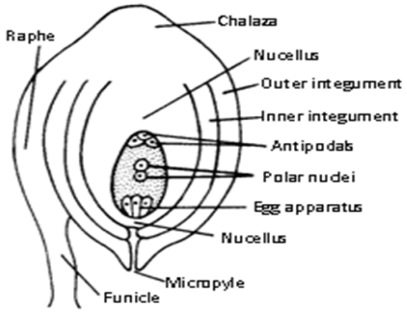


Fig.9.2, Structure of a typical anatropous ovule before fertilization showing fully developed embryo sac

INTEGUMENTS: These are outer most multicellular layer of the ovule. On the basis of number of integuments, ovules are of following types:

1. **Unitegmic ovule:** In this ovule, only one integument is present e.g. Gymnosperm (*Cycas*, *Pinus*), and gamopetalous family e.g. Compositae, Solanaceae etc.
2. **Bitegmic ovules:** In this type, two integuments are present e.g. Polypetalous angiosperm (Cruciferae, Malvaceae, Cucurbitaceae) and monocots. It is of common type and has been found in 208 families.
3. **Ategmic ovules:** These types of ovules are without any integuments e.g. Loranthaceae and Santalaceae (In the parasite like *Cuscuta and Loranthus*), *Santalum, Liriosma, Olax* etc.

Nucellus: Nucellus forms the main part of the ovule which is made up of parenchymatous tissue. Nucellus is also called as megasporangium proper. Nucellus provides nutrition to developing embryo sac.

1. **Tenuinucellate nucellus:** The ovule having less develop nucellus is called tenuinucellate nucellus e.g. gamopetlous family (compositae, malvaceae, solanaceae, rubiaceae).
2. **Crassinucellate nucellus:** The ovule having well develop nucellus is called crassinucellate nucellus e.g. in polypetlous families and monocots (most of the angiosperms).

Types of Ovules: There are six different kinds of ovules:

1. **Orthotropous (Atropous):** It is the primitive type of ovule. In this funicle, chalaza and micropyle lie in one vertical plane. It is erect or straight ovule e.g. all gymnosperm, *polygonum, Piper nigrum, Piper betel.* (Polygonaceae, Piperaceae, Casuarinaceae, Urticaceae) etc.
2. **Anatropous or inverted ovules:** It is the most common type of ovules, occurring in more than 82% of the angiospermic families. The body of ovule rotated at 180º and hilum at 90º to micropyle. The funicle and micropyle lay side by side e.g. compositae, solanaceae etc.
3. **Campylotropous:** This type of ovule is curved e.g. common type in leguminosae and caryophylaceae, chenopodiaceae (*Chenopodium*), capparidaceae (*Capparis)* and some members of brassicaceae like Mustards and *Capsella***.**
4. **Hemitropous/Hemianatropous:** It is transverse ovule. In this body of ovule is at right angle to funicles. Body of ovule turned through 90º and micropyle and chalaza are in straight line e.g. Ranunculaceae, Primulaceae and some members of Brassicaceae.
5. **Amphitropous (Horse shoe shaped):** In this body of ovule and embryo sac both are curved, micropyle, chalaza and funicles lay near each other e.g. Butamaceae (*Butamus*), Loganiaceae, Alismaceae, Papaveraceae.
6. **Circinotropous:** In this funicles coiled around the ovule. It is most complex type e.g. *Cactaceae (Phyllocactus, Opuntia) Plumbaginaceae (Plumbago*).

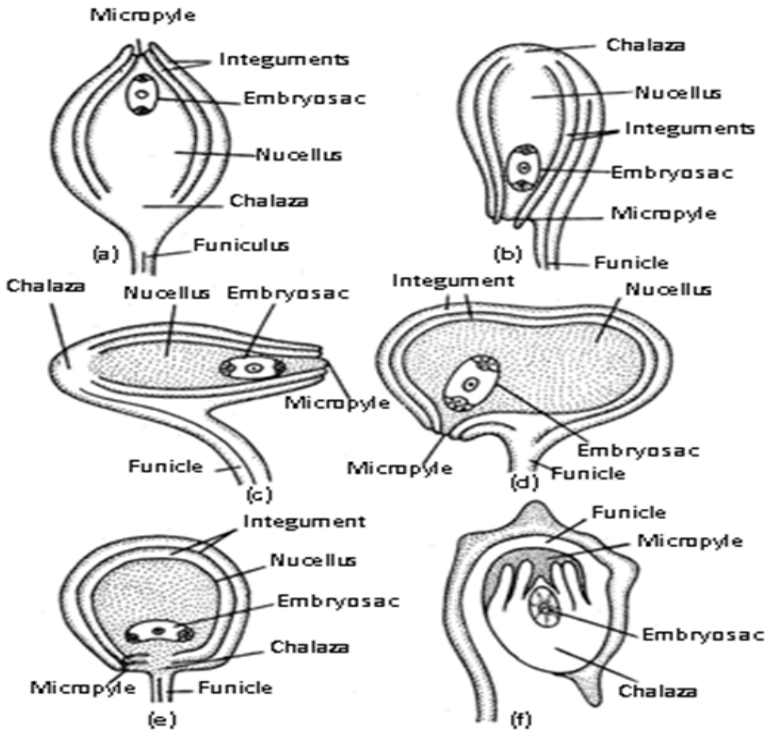


Fig. 9.3, Types of ovule: a. Orthotropous, b. Anatropous, c. Hemianatropous, d. Campylotropous, e. Amphitropous, f. Circinotropous

Some Special Structure Associated With Ovule

a) **Endothelium:** It is modified innermost layer of integuments in some unitegmic ovule. Its cells are radially elongated, polyploid, and full of starch and fats. It provides nutrition to embryo sac. It is generally single layered but 10-12 layered endotheliums are present in family compositae. It is present in 65 families of dicots. It is similar to tapetum of anther and also called integumentary tapetum.

b) **Obturator:** It is the group of unicellular or multicellular hairs originating from placenta (e.g. Euphorbiaceae, Cuscutaceae) and from funiculus (e.g. Lamiaceae, Magnoliaceae, Acanthaceae). It helps in attraction of pollen tube by secreting some chemicals. It shrinks after the act of fertilization.

c) **Nucellar beak:** In some cases, nucellar comes out in the form of a beak like structure e.g. Euphorbiaceae, Cucurbitaceae, Polygonaceae.

d) **Hypostase:** It is the group of lignified cells below embryo sac in the nucellus. The term hypostase coined by Van Tieghem (1901). It prevents shrinkage of embryo sac into the base and also maintains water balance. It is found in Amaryllidaceae, Liliaceae, Euphorbiaceae and Zingiberaceae.

e) **Caruncle:** It is formed by proliferation of cells of outer integument in micropylar region. Its function is to absorb water during seed germination.

f) In some plants of family Cactaceae, in between two integuments, a prominent air space is present at chalazal end.

Developoment of Female Gametophyte (Embryo Sac)

Megasporogenesis and megagametogensis

Megasporogenesis: In the ovule, some hypodermal or sub-hypodermal cells of nucellus become big with dense cytoplasm and prominent nucleus which behave like archesporial initial which by periclinal division forms parietal cell and sporogenous cell (2n). The parietal cell degenerates and the

sporogenous cell differentiates as megaspore mother cell (2n). Megaspore mother cell undergoes meiosis to form four haploid megaspores (n) which occur in the form of linear tetrad. Formation of megaspores from megaspore mother cell is known as megasporogenesis.

Megagametogenesis: Out of four megaspores, three towards micropylar end degenerate and the fourth megaspore at chalazal end remains functional. Nucleus of functional megaspore undergoes three free nuclear mitotic divisions to form eight nuclei (n), four towards micropylar end and four towards chalazal end. Out of four nuclei at each pole, one nucleus (polar nucleus) moves to the centre which form diploid secondary nucleus. Three nuclei at micropylar end form egg apparatus (one egg cell and two synergids - all haploid). Three nuclei at chalazal end form antipodal cells (all haploid and vestigial). In this way, functional megaspore develops into mature embryo sac. Formation of egg from functional megaspore through three free nuclear divisions is known as megagametogenesis. Mature organized embryo sac at the time of fertilization is 7-celled and 8-nucleated structure which is monosporic in nature and is known as polygonum type of embryo sac, because it was discovered by Strassburger for the first time in *Polygonum*. Polygonum type of embryo sac is most simple, most primitive and normal type of embryo sac. In angiosperm, embryo sac is fully developed female gametophyte.

Note: In embryo sac, all the cells are haploid except secondary nucleus (n + n). In *Balanophora*, one megaspore of micropylar end is functional. In *Casuarina*, all or any one of four megaspores may be functional**.** The development of female gametophyte is completely endosporous i.e. within the megaspore.

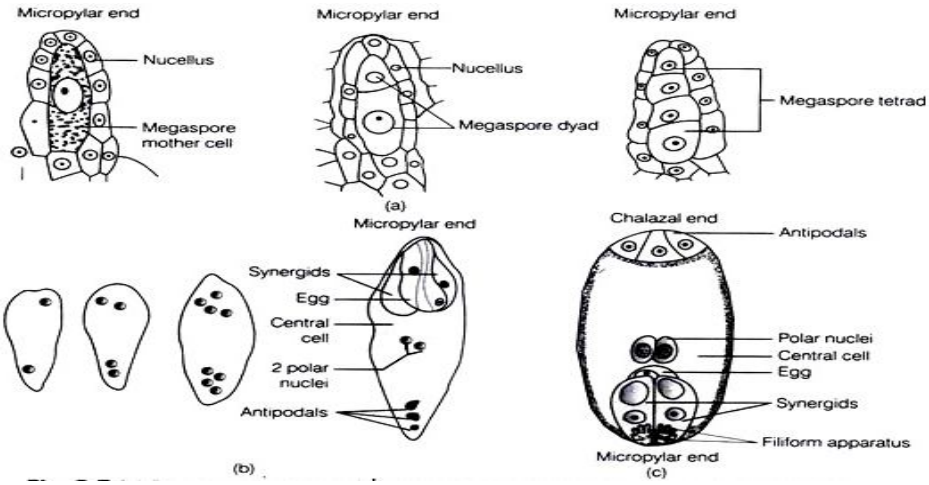


Fig. 9.4, Successive stages of Megasporogenesis and development of female gametophyte

(a) parts of the ovule showing a large megaspore mother cell, a dyad and a tetrad of megaspore, (b) 2, 4 and 8 nucleate stages of embryo sac and a mature embryo sac, (c) A diagrammatic representation of the mature embryo sac

Table -1, Difference between microsporogenesis and megasporogenesis

Microsporogenesis		**Megasporogenesis**
1.	It is the process of the formation of microspore tetrads from a microspore mother cell through meiosis.	It is the process of the formation of the four megaspores from a megaspore mother cell in the region of the nucellus through meiosis
2.	It occurs inside the pollen sac of the anther.	It occurs inside the ovule.

Structure of embryo sac: The most common type of embryo sac in angiosperm is 8-nucleated and 7-celled. It is called polygonum type of embryo sac since it was studied in *Polygonum divaricatum* by Strassburger. It has three main parts:

1. **Egg apparatus:** It is the group three cells present toward the micropyle (1 egg cell and 2 synergids). Synergids contain finger like projections called filiform apparatus which may help in absorption and conduction of food materials from nucellus into the embryo sac. Synergids also act as the director for the entry of pollen tube into embryo sac. Filliform apparatus is rich in polysaccharides.
2. **Antipodal cells:** A group of three cells present towards chalazal end of the embryo sac.
3. **Central cells:** It is the central large cell with two polar nuclei. They unite to form a diploid nucleus. It is called secondary nucleus.

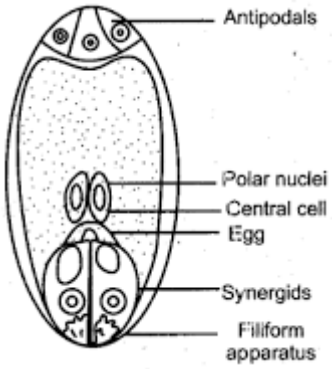


Fig. 9.5, Mature embryo sac of an angiosperm

Types of Development of Embryo Sac: According to **P. Maheshwari,** the following three types of embryo sacs have been recognized on the basis of (1) the number of megaspore contributing in formation of embryo sac, (2) the number of mitotic division takes place in functional megaspore and (3) the organization of nuclei in embryo sac. These three are as follows:

1. **In monosporic embryo sac** only one megaspore takes part.
2. **In bi-sporic embryo sac** two megaspores take part.
3. **In tetra-sporic embryo sac**, all four megaspore take part.

1. **Monosporic embryo sac:** A monosporic embryo sac develops from a single megaspore and as such all the nuclei present in this type of embryo sac are genetically alike. Mono-sporic embryo sacs are of the following two types:-

a) **Monosporic 8- nucleate or Polygonum type of embryo sac:** (8N + 7C), 3+2+3 nuclear arrangement, (endosperm – 3n)**.** It was first time studied by Strassburger (1879) in *Polygonum divaricum* and hence the name Polygonum. It occurs in 70% of angiosperms. At chalazal end, megaspore is functional and total three mitotic divisions (3 free nuclear divisions) occur in the nucleus of megaspore producing eight nuclei. Out of which three nuclei at chalazal end form antipodal cells, three nuclei at micropylar end form egg apparatus and two nuclei in the centre as polar nuclei and thus mature embryo sac has 8 nuclei and 7 cells. This is the simplest and most primitive and most common type of embryo sac in angiosperm.

b) **Oenothera type embryo sac:** (4N + 4C), 3+1 nuclear arrangement, (endosperm – 2n).

It was first observed by Holfmeister (1849) in *Oenothera lamarkiana* (Characteristic feature of family Onagraceae)**.** Functional megaspore is at micropylar end and only two free nuclear divisions occur in it and hence four nuclei are formed, three are arranged as egg apparatus with one in the centre as polar nucleus. No antipodal cells are formed in this case and thus mature embryo sac has 4 nuclei and 4 cells.

2. **Bisporic type of embryo sac:** In this case, first meiosis in megaspore mother cell is followed by cell division and a dyad is formed. Now one of dyad degenerate and another take part in embryo sac development by two successive mitotic division and form 8 nucleate and 7 celled embryo sac like polygonum type. Based on the position of dyad takes part in the formation of embryo sac, it is of two types:

a) **Allium type embryo sac:** (8N + 7C), 3+2+3 nuclear arrangement, (endosperm - 3n). It was discovered by Strassburger (1879) in *Allium fistulosum*. In this case, micropylar dyad degenerate and chalazal dyad takes part in embryo sac development by two successive free nuclear divisions forming eight nuclei with the following arrangements; three forms egg apparatus, two polar nuclei and three antipodal cells. *Allium* type is found in podostemaceae, Blanorophoraceae, Loranthaceae (all dicots) and Liliaceae, Amaryllidaceae, Orchidaceae, Butamaceae (all monocots)

b) **Endymion type embryo sac:** (8N+7C), 3+2+3 nuclear arrangement (endosperm – 3n). It was discovered by Fagerlind (1944) in *Endymion hispericus*. In this dyad of micropylar end takes part in embryo sac development and the rest is same as in *Allium* type.

3. **Tetrasporic type of embryo sac:** In this case, meiotic division in megaspore mother cell does not follow wall formation and the resultant is the four nucleate, uni-celled called **coenomegaspore**. All the four nuclei take part in embryo sac development. On the basis of number of division in coenomegaspores, tetra-sporic embryo sac may be of the following types:

a) **Adoxa type:** (8N + 7C), 3+2+3 nuclear arrangement, (endosperm – 3n). It was discovered by Johnson in *Adoxa moschatellina*. Only one free mitotic division occurs in coenomegaspore, which produces 8 nuclei.

b) **Plumbago type:** (5N+ 2C), 1+4 nuclear arrangement, (endosperm – 5n). Haupt (1934) discovered it in *Plumbago capensis*. One mitotic free division occurs in coenomegaspore producing 8 nuclei. Two nuclei arranged on each pole (on four sides). One nucleus from each pole migrates to centre and form four nucleate central cells (polar nuclei). One nucleus at micropylar end behaves as egg and the remaining nuclei degenerate.

c) **Penaea type:** (16N+ 13C), 3+4+3+3+3 nuclear arrangement, (endosperm – 5n). It was discovered by Stephen in *Penaea*. In each nucleus of coenomeagspore, two mitotic free division occur which lead the formation of 16 nuclei (4 nuclei at each corner)**.** One nucleus from each corner migrates to centre forming 4 polar nuclei. Remaining three at micropylar end forms egg apparatus (one egg + two synergids). Remaining three at chalazal end forms antipodal cells. While remaining 6 nuclei (three at each lateral side) form parietal cells.

d) **Peperomia type embryo sac:** (16N+9C), 2+8+6 nuclear arrangement, (endosperm – 9n). It was discovered by Campbell (1899-1901) in *Peporemia pellucid.* Nuclear division is same as in Penaea type. Only arrangement of nuclei is different. Here two nuclei (one synergid + one egg) are at micropylar end, 8 nuclei as polar nuclei and 6 nuclei arranged as antipodal cells at chalazal end.

e) **Drusa type embryo sac:** (16N+15C), 3+2+11 nuclear arrangement, (endosperm – 3n). It was discovered by Hakansson in *Drusa oppositifolia.* Nuclear division is same as in *Penaea* type. 3 nuclei at micropylar end behave as egg apparatus, two nuclei as polar and 11 nuclei at chalazal end form antipodal cells.

4. **Tetra-sporic special types of embryo sac development**

a) **Fritillaria type embryo sac: (16N + 8C), 3+4+9** nuclear arrangement, **(endosperm – 5n).** Megaspore mother cell forms 4 megaspores after meiosis. One migrates towards the micropylar end and remaining three moves to chalazal pole where they fuse and form triploid nuclei. Two successive mitotic divisions occur in both these nuclei forming 4 cells at micropylar pole (haploid) and four cells at chalazal pole (each triploid). One haploid nucleus from micropylar end

and one triploid nucleus from chalazal pole migrate to centre and organize as polar nuclei (one haploid + one triploid). Three haploid cells at micropylar region forms egg apparatus**.** Three triploid cells at chalazal end form antipodal cells.

b) **Plumbagella type embryo sac: (8N + 4C), 1+4+3** nuclear arrangement, **(endosperm – 5n).** Meiosis occurs in megaspore mother cell which produces 4 nuclei; one migrates to micropylar region and three moves to chalazal pole where they fuse forming triploid nuclei. Now **one mitotic division occurs** in these two nuclei producing two haploid cells at micropylar end and two triploid cells at chalazal end. In the rearrangement of nuclei, one haploid cell from micropylar end and one triploid cell from chalazal end migrate to centre and forming there polar nuclei. One haploid cell at micropylar end behaves as egg, **(synergids absent**). One triploid cell at chalazal end behaves as antipodal cell.

Note: Generally endosperm is triploid but it is 2n in *Oenothera*, 9n in *Pepromea*, 5n in *Fritillaria.*

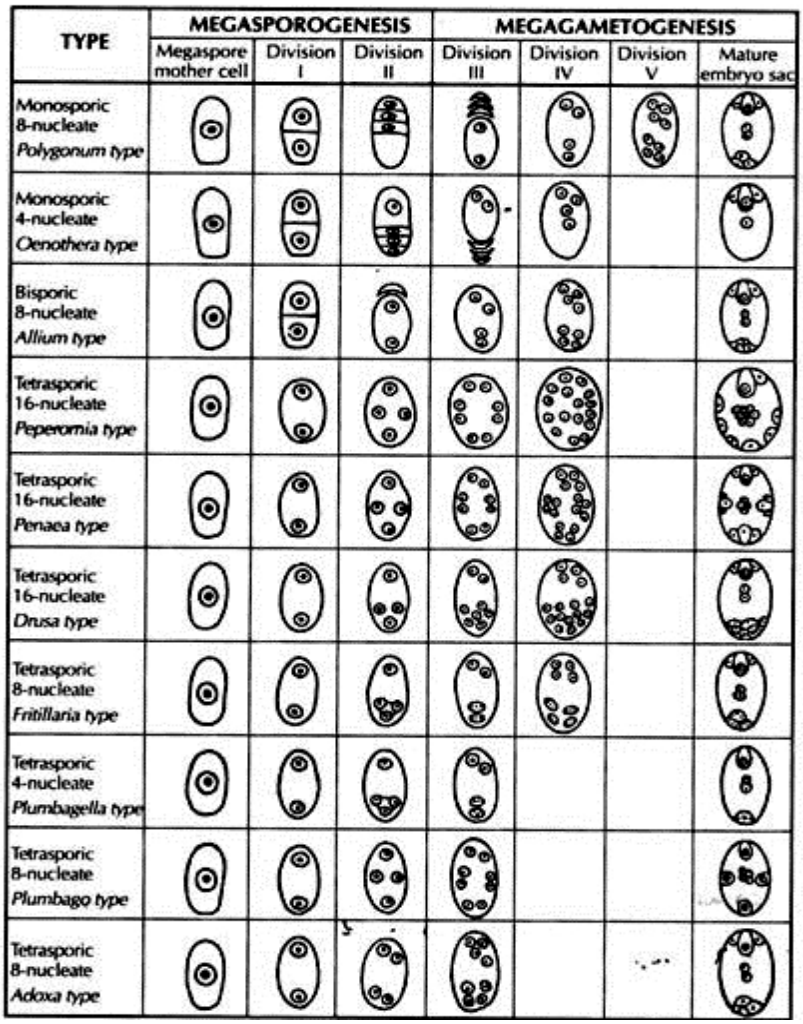


Fig. 9.6, Development of different types of embryo sac in angiosperms (After Maheshwari)

(Micropyle above in all illustrations)

Chapter-10

Pollination

Sexual reproduction involves the mating of male and female gametes which are produced in anther and carpel of the flower respectively. So, the transfer of pollen from anther to the stigma of carpel is necessary step for sexual reproduction. The transfer of pollen grains from anther to stigma is called pollination.

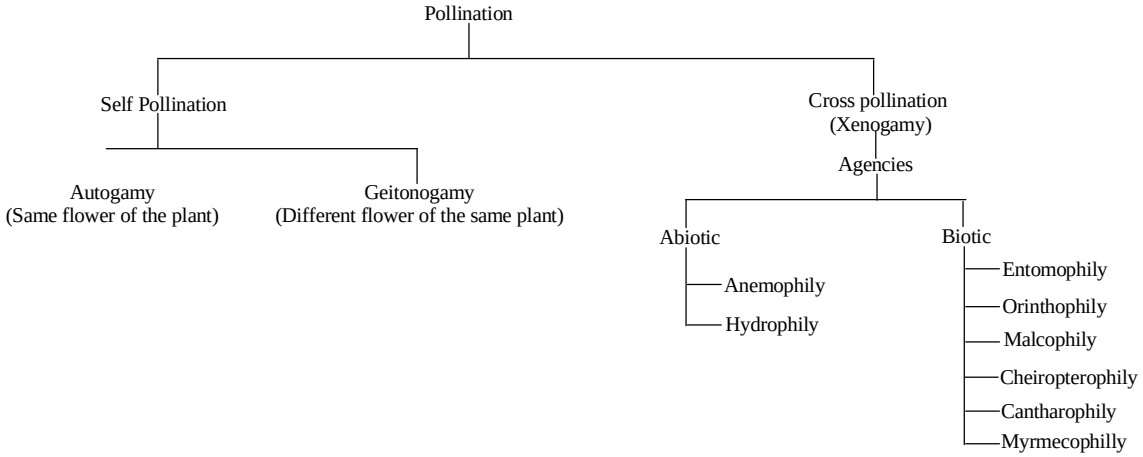


Fig. 10.1, Types of pollination

Types of pollination: Based on the destination of pollen grains, two types of pollination are recognized:

A. **Self pollination:** The transfer of pollen grains from anther to the stigma of same or genetically similar flower is called self pollination. Self pollination is of two types:

a) **Autogamy** is the transfer of pollens from anther to the stigma of the same flower e.g. *Vinca, Mirabilis* (4, o,clock), *Commelina,* potato etc.

b) **Geitonogamy** is the transfer of pollens from the anther of one flower to the stigma of another flower of the same plant e.g. Maize etc.

B. **Cross pollination (Allogamy):** It is the process of transfer of pollens from the anther to stigma of genetically different flower. This process is also termed as xenogmay e.g. *Valisnaria, Salvia* etc.

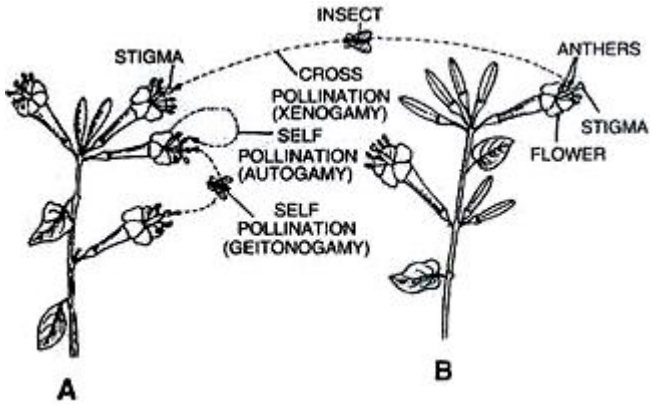


Fig 10.2, Self and Cross pollination

Contrivances of autogamy or **adaptation of autogamy:** Autogamy takes place under following essential favouring conditions:

1. **Bisexuality or Hermaphroditism:** It is the only bisexual flowers where self pollination can take place. Self-pollination is a rule in bisexual flowers.
2. **Homogamy:** When both anther and stigma of bisexual flowers matures at the same time e.g. rice, wheat, potatoes etc.
3. **Cleistogamy:** In this case, flower is never open in their life span, so self pollination is obligatory. In some plants e.g. *Viola, Oxalis, Commelina, Arachis,* there are two types of flowers, chasmogamous and cleistogamous. Chasmogamous flowers are normal open flowers with exposed anther and stigma. Cleistogamous flowers are closed with stigma and anther lie close to each other. It is invariably autogamous which show assured seed set.
4. **Bud pollination:** Anthers and stigma of bisexual flowers mature before opening of bud and thus self pollination takes place at the time of bud stage e.g. Pea, Wheat etc.

Note: *Commelina benghalensis* bears both chasmogamous as well as cleistogamous flowers. Plants bearing both types of flowers are called as chasmocleistogamous flowers.

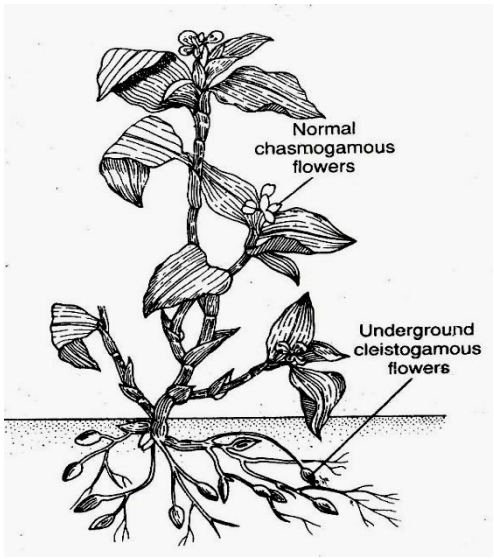


Fig. 10.3, Showing chasmogamous and cleistogamous flowers

Contrivances to ensure cross pollination

1. **Dicliny (Unisexuality)**: Cross pollination is a rule in unisexual flowers. There may be two types of unisexuality i.e. Monoecious plants (male and female flower born on the same plants e.g. maize, cucurbits, castor etc. and dioecious plants (male and female flowers are born on different plants e.g. *Carica papaya* etc.*).*
2. **Dichogamy:** This is the maturation of anthers and stigma at different times. There are two conditions of dichogamy:

a) **Protandary:** Anthers mature earlier than the stigma of the same flower e.g. Cotton, Sunflower, *Salvia, Jasminum, Foeniculum,* Family Umblliferae *etc.*

b) **Protogyny:** Stigma matures earlier than the anthers of the same flower e.g. *Ficus, Magnolia, Aristolochia, Bajra, Impatiens, Anona, Plantago, Ranunculus, Mirabilis jalapa etc.*

3. **Herkogamy:** It is the condition when some physical barrier is present between male and female reproductive parts e.g. in members of carophyllaceae, the stigma projects beyond stamens. In *Gloriosa,* anthers dehisce at distance from stigma. In Orchids and members of Ascelpiadaceae (e.g. *Calotropis*), pollen remain aggregated in pollinia.
4. **Heterostyly:** It is the condition in which styles and filaments are of different heights e.g. *Lythrum, Linum, Jasminum, Primula* etc. Plants are dimorphic i.e. bears two types of flowers e.g.

Oxalis, Primula, Oldenlandia etc. *Linum* and *Lythrum* show trimorphism. *Primula vulgaris* show **dimorphism (di-styly**) in which pin eyed or long styled possess long style, long stigmatic papillae, short stamen and small pollen; and thrum eyed or short styled flower possess short style, small stigmatic papillae, long stamens and large pollen.

5. **Self sterility or self incompatibility:** Due to some genetic reason, some time stigma and stamens of a flower mature at the same time but pollen become incapable of fertilization e.g. *Malva, Orchids, Nicotiana, Potato, Solanum, Petunia* etc.

Pollen pre-potency: When pollen from other flowers is more effective and grows faster than its own pollen, this condition is termed as pollen pre-potency. This is seen in apple.

Agencies of cross pollination: About 80% of all plant pollination is biotic. Of the 20% of abiotically pollinated species, 98% is by wind and 2% by water. The agencies are as:

A. **Anemophily:** The pollination by wind is called anemophily. It was first demonstrated by Sprengel (1793) e.g. Grasses, Wheat, Maize, Rice, Barley, Palms, coconut, Oaks, Cotton woods, Date palm, Salix, Betula, Pinus (contain winged pollen grain), Cycas etc. The characteristics of wind pollinated flowers are the presence of light and non sticky pollen grains, well exposed stamens, large and feathery stigma for easy trapping of pollen grains, single ovule in single ovary and numerous flowers packed into inflorescence.

B. **Hydrophilly:** Pollination with the help of water is called hydrophilly. When pollination occur in submerged plant, it is called hypohydrophilly e.g. Ceratophyllum, Najas, Zostera. Pollination in floating hydrophytes is called epihydrophilly e.g. *Potamogeton, Vallisnaria, Myriophyllum. Vallisnaria spiralis* (ribbon weed**)** is a classic example of these. It is classic in the sense that plant is submerged but at the maturity flower detached from plant and float on the surface of water body. The characteristics of hydrophillous flower are the presence of small and inconspicuous flowers; odor, nectar and color absent, pollen grains are small, light, ribbon like, non sticky and coated by wax and female flower with long stalk.

C. **Animal pollination (Zoophily):** A range of animals are used as pollinating agents e.g. Wasps, ants, moths, birds, lemur, rodents, reptiles etc. Zoophily is of the following types:

1. **Entomophily:** It is the pollination by insects. The pollens are rough and sticky, insects attracted by color/ scent/nectar. Some common insects are bees (commonest), butterflies, moth, wasp, beetles etc. Bees handle 80% of all pollination done by insects.

2. **Orinthophily:** Pollination by birds is known as orinthophily. The characteristics of bird pollinated flowers are large size, brightly colored, odourless and large amount of mucilaginous nector. Besides these the corolla is generally tubular (*Nicotiana glauca, Bignonia),* cup shaped (*Callistemon*) or urn shaped (some members of Ericaceae). *Strelitzia reginae* and *Aloe vera* pollinated by sun birds; *Begonia* by humming birds, *Salmelia* is by crow and mainas etc.

3. **Cheiropterophilly:** It is the pollination by bats. It occurs mostly in tropics. Flowers born singly or in cluster but quite away from the branches and highly fragrant which facilitate bats in the night for pollination e.g. *Kigellia pinnata, Anthocephalous, Adansonia digitata* etc. Bat pollinated flowers have parts which are usually fleshy and sweet in test e.g. Kadam *(Anthocephalous cadamba).*

4. **Malcophily:** It is the pollination by snails and slugs e.g. *Colocasia, Lemna* and some member of family Poaceae.

5. **Myrmecophilly:** It is the pollination by means of ants e.g. members of family Mimosaceae (*Acacia, babool, kikar* etc.) and Rubiaceae.

6. **Cantharophily:** It is the pollination by means of beetles.

Table: 10.1, Basic Pollination Syndrome Characters

Flower	Bats	Bees	Beetles	Birds	Butterflies	Flies	wind
Colour	Dull white, green, purple	Bright white, yellow blue	Dull white, green	Orange, red, white	Orange, red, purple	Pale and dull to dark brown Or purple	Dull green or brown
Odour	Strong, fruity	Fresh, mild, pleasant	Fruity, juicy	None	Spicy	Putrid	None
Shape	Regular, bowl shaped, closed during day	Shallow, landing platform, tubular	Large, bowl like	Large, funnel like, strong perch support but not landing platform	Narrow, tube, wide landing pad	Shallow funnel like or trap like	Regular, small stigma exerted, petals absent or reduced
Bloom time	Night	Day	Day	Day	Day	Day and night	Any time
Nectar	Abundant, somewhat hidden	Usually present	Some time present, not hidden	Ample, deeply hidden	Ample, deeply hidden	Usually absent	None

Pollen-Pistil Interaction: Only compatible pollen is accepted by stigma while others are rejected either by preventing pollen germination or pollen tube growth. Pollen is released at 2-celled or 3-celled stage. If it is released at 2-celled stage, further division of pollen tube takes place during pollen tube growth. 3-celled pollen grain carries male gametes at the beginning itself. Entry of pollen tube into ovule is generally occurs through the micropyle and synergids. The filiform apparatus guides the pollen tube into micropylar part of synergids.

Some Important Points about Pollination

1. Beetles, flies, bees visit flower during day (diurnal), attracted towards flower by color and nector. Bees are color blind for red and are attracted towards the yellow, violet and purple color.
2. Moths pollinate flower in the night (nocturnal). Nocturnal flowers are dull in color and highly fragrant.
3. Unpleasant smell of some flower attract the flies for pollination e.g. *Arum* (human dung), *Rafflesia* (Rotten meat), *Aristolochia* (decaying tobacco and humus). *Aristolochia*, *Arum* and *Ceropegia* have developed fly trap mechanism for pollination.
4. In ***Salvia*** (Sage plant–family Labiatae) – pollination by bees occurs by turn pipe mechanism or lever mechanism.
5. In ***Ficus* species** having hypanthodium inflorescence, trap door mechanism occurs for pollination.
6. **Piston mechanism** of pollination occurs in *Centaurea.*
7. **Bristle mechanism of pollination occurs in** *Pinguicula alpine.*
8. ***Calotropis*** shows **translator or clip mechanism** for pollination.
9. *Ophrys speculum* (orchid flower) resemble the wasp *(Calpa aurea)* in shape and pseudocoupulation occurs which causes the pollination in *Ophrys*. In addition these flowers emit a chemical pheromone that resembles the odor produced by female wasp for sexual attraction**.**

10. **Mutualism of pollination:** In *Yucca* (family Liliaceae), pollination occur by moth called *Tageticula* (yucca moth). *Tageticula* can not complete life cycle without *Yucca* and *Yucca* has no other pollinator. There is an obligate symbiotic relationship. Both the species can not complete their life cycle without each other, moth deposits its eggs in the locule of the ovary, and the flower in turn, gets pollinated by the moth. The larvae of the moth come out of the eggs as the seeds start developing.
11. Pollination in *Erythrina* takes place by means of crows and squirrels.
12. Flowers of *Aristolochia clematis* show pit fall mechanism. Fly can crawl down but cannot get out because of the hairs pointed down.

Chapter-11

Fertilization

Fertilization was first of all reported by Strasburger (1884) in *Monotropa* plant. The fusion of male gamete with female gamete (egg) is called fertilization. If the male gamete brought to the egg by pollen tube, the phenomenon is called as siphonogamy. Thus fertilization in angiosperms is siphonogamous type. Process of fertilization can be studied in the following steps:

Germination of pollen on stigma: Pollen grain lands on stigma and germinate by absorbing stigmatic secretions which contain sugar, gums, lipids, resin and other liquid and swells up. Exine ruptures and comes out in the form of pollen tube. As it was earlier said that the pollination occurs at two celled stage of male gametophyte, now after landing on stigma, generative cells divide mitotically to produce two male gametes. Vegetative cells (tube cell) help in pollen tube formation. Generally one pollen tube arises from single pollen and this condition is known as monosiphonous condition. In certain cases, many pollen tubes arise from single pollen and now this condition is known as polysiphonous condition e.g. in Malvaceae, Cucurbitaceae and Companulaceae more than one pollen tube is formed but only one of them is functional.

Types of style: On the basis of movement of pollen tube through style, styles are of three types.

1. **Open style:** Style is hollow in this case and pollen tube creeps on the surface of special lining called stylar canal (ectotrophic) e.g. most of the monocots and Papaveraceae
2. **Half closed style:** In this case, style is semi solid e.g. Cactaceae
3. **Closed/Solid style:** In this case, there is no open channel, pollen tubes moves through intercellular spaces present e.g. *Dhatura,* Cotton (mostly dicot). Pectinase and hydrolytic enzymes secreted from the pollen tube provide the way.

Note: Whatever it may be but pollen tube absorbs nutrition from styler tissue. Nutrition is present in the form of pectic tissue which dissolved by enzymes secreted by pollen tube. Thus developing male gametophyte is parasite over sporophytic tissue of style. The reason for pollen tube growth towards the ovary is perhaps due to chemotropic stimulus (Calcium-boron-inositol sugar complex).

Entry of pollen tube into ovule: Pollen tube enters the ovule in either of three ways:

1. **Porogamy:** The process of entry of pollen tube through micropyle is called porogamy. It is the most common type.
2. **Chalazogamy:** The process of entry of pollen through chalaza is called chalazogamy e.g. *Betula, Casuarina, Juglans* etc. It was first observed by *Treub (1891) in Casuarina.*
3. **Mesogamy:** The entry of pollen tube through integuments or funiculus is called mesogamy e.g. through integuments in *Cucurbita, Populus* or through funiculus in *Pistacia*.

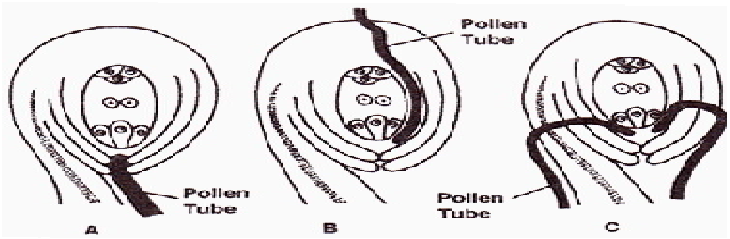


Fig. 11.1, Entry of pollen tube into ovule

A-porogamy; B- chalazogamy; C-mesogamy

Note: Whatever it may be the way of entry to ovule but pollen tube always enters the embryo sac at the micropyler end. Pollen tube passes between wall of egg cell and one of the synergid cells or between the wall of egg cell and embryo sac or through the path formed by degenerating synergid. As

a rule one synergid destroyed and other remain intact until sometime after wards. Filiform apparatus of synergids release some chemical to attract pollen tube i.e. chemotactic stimulus for guiding pollen tube towards egg. Filliform apparatus is finger like projection rich in polysaccharides.

Discharge of male gamete from pollen tube: Pollen tube burst by absorbing hydrolytic substances secreted by degenerating synergid and release its contents which include two male gametes. There are three way of discharge of gametes:

1. In one case, two terminal opening produced in the pollen tube and one gamete discharged through one opening.
2. In the second case, the apex of pollen tube burst and releases both gametes at a time.
3. In the third case, tip of the pollen tube produced two branches. One directed towards the egg while another towards polar nuclei. Later the apical end of each branch burst and release gamete.

Process of fertilization: Inside the embryo sac, one male gamete fuses with the egg to form zygote (2n), this process is known as syngamy or generative fertilization. Zygote later develops into embryo. The second male gamete fuses with two polar nuclei or secondary nucleus to form triploid primary endosperm nucleus. This process is known as triple fusion or vegetative fertilization. Primary endosperm nucleus later develops into endosperm. The occurrence of syngamy and triple fusion simultaneously in angiosperms is called **double fertilization**. Syngamy was discovered by Strasburger while triple fusion and double fertilization was discovered by Nawaschin in *Fritilaria* and *Lilium*. Double fertilization is unique feature of angiosperms. Antipodal and synergid cells degenerate after fertilization. Total number of nuclei involve in double fertilization is five i.e. 2 nuclei in syngamy + 3 nuclei in triple fusion. Gerassimova (1930) reported that triple fusion occurs more quickly than syngamy.

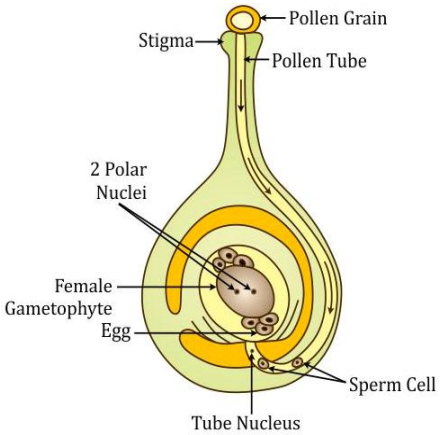


Fig.11.2, Showing process of fertilization (Syngamy)

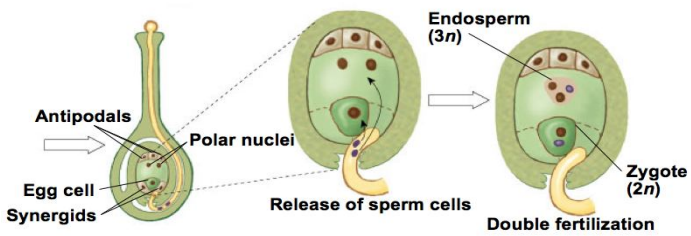


Fig. 11.3, Showing process of double fertilization

Post fertilization changes: After fertilization ovary stores food materials and develops into fruit and the ovules develop into seeds. Primary endosperm nucleus develops into endosperm. The outer and inner integuments of the ovule develop into outer and inner seed coats called testa and tegmen respectively. The remaining portions of the nucellus change into perisperm. Antipodal and synergids

degenerate. Funicle becomes the stalk of the seed. Micropyle becomes the opening of the seed (micropyle of seed). Stigma and style become dry and drops off.

Perisperm: Perisperm is remnant of nucellus. Seeds having perisperm are called perispermic seeds e.g. *Mirabilis jalapa, Nymphaea, Zingiber, Portulaca, Canna, Capparis etc.* Edible part of the coffee seed is perisperm.

Polyspermy: Some times more than two male gametes reach in one embryo sac. This phenomenon is known as Polyspermy. Polyspermy may be due to entry of more than one pollen tube inside embryo sac or due to presence of more than two male gametes in single pollen tube. In this condition, egg may fertilized by more than one gametes to form polyploid zygote or the extra male gametes fuse with synergids or antipodals etc. to give rise to polyembryony.

Heterofertilization: Egg fuses with male gamete of one pollen tube but the polar nuclei fuse with male gamete of another pollen tube.

Chapter-12

Endosperm

In gymnosperm, it is haploid and develops before fertilization. So, it is pre-fertilization tissue in gymnosperms. In angiosperms, it is generally triploid, a product of double fertilization and developed after fertilization. So in Angiosperms, it is post fertilization tissues. No endosperms are formed in Podostomaceae, Orchidaceae and Trapaceae. In these cases, triple fusion is completed but the fusion product either degenerate or undergo only one or two division. Endosperm is nutritive tissue which provides nourishment to the developing embryo in seed plant.

Types of Endospers: There are three types of endosperms (1) Nuclear type (2) Cellular type (3) Helobial type. These three types can be distinguished only after first division of primary endosperm nucleus.

(1) **Nuclear type:** It is most common and most primitive type and reported in 161 families out of 288 families for which record is available. The first and subsequent division of primary endosperm nucleus is not followed by wall formation. Thus free nuclei remain in the cytoplasm of embryo sac e.g. most common in Polypetlae, Cotton, *Zea mays, Arachis, Citrus, Malva, Primula, Capsella* etc.

(2) **Cellular type:** It is reported in 72 families of mostly dicots. The primary endosperm nucleus divides by mitosis and each subsequent division followed by cell wall formation e.g. *Adoxa, Verbascum, Magnolia, Impatiens, Datura* etc.

(3) **Helobial type:** It is reported in about 17 families particularly of monocots. It is intermediate between nuclear and cellular type. Here first division in primary endosperm nucleus is followed by wall formation but later on, the divisions are free nuclear e.g. in families of order helobiales like Alismaceae, Butomaceae, Hydrocharitaceae, Najadaceae etc. *Asphodelus* is a common example.

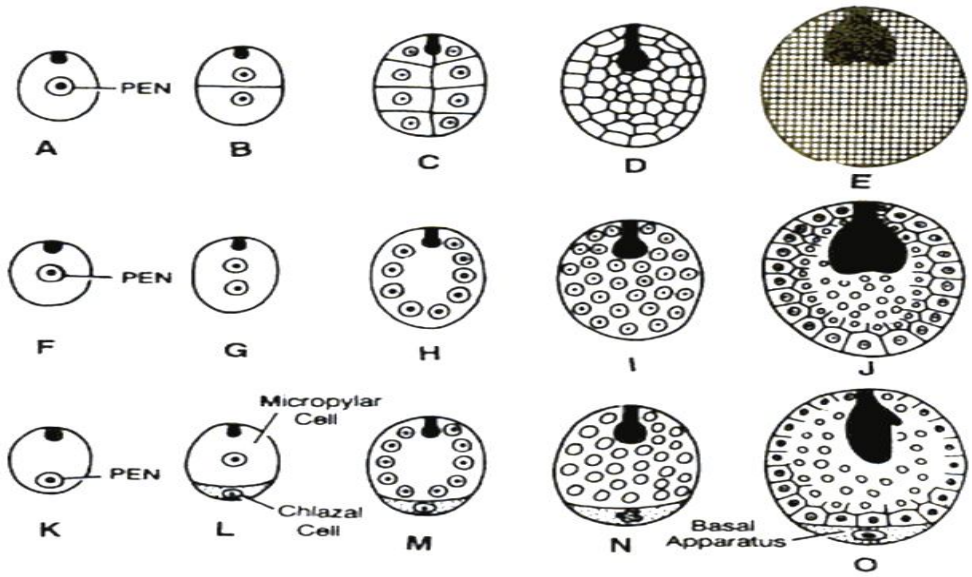


Fig. 12.1, Kinds of endosperm

A-E. Development of cellular endosperm, F-J. Development of nuclear endosperm, K-O. Development of helobial endosperm, (PEN- Primary Endosperm Nucleus)

Functions of endosperms

1. It is nutritive in function and store carbohydrate, protein, fats etc. which provide nutrition to developing embryo.
2. Coconut milk (liquid endosperm of coconut) contains auxin, cytokinin and gibberelic acid and induces cytokinesis.

3. Young endosperm of maize (milky) also induces cytokinesis and a very common cytokinin known as zeatin is extracted from milky endosperm of maize. Mature endosperm does not have stimulatory effect.

Special types of endosperm

1. **Ruminant endosperm:** In this type of endosperm, surface of endosperm is uneven, having ridges and furrows due to invagination of nucellus or integuments into endosperm e.g. *Passiflora* and *Cocoloba*. It is reported in 32 families of angiosperm e.g. Rubiaceae, Annonaceae, Aristolochiaceae, Palmaceae, Myristicaceae, Apocyanaceae, Polygonaceae etc.

2. **Mosaic endosperm:** In this type of endosperm, the tissue of endosperm is not homogenous but there are patches of different color i.e. heterogenous e.g. *Zea mays* where distinct yellow and white patches occur and also Webber in 1900 observed some portion as starchy and some portion sugary. Webber suggested that mosaic pattern of endosperm is due to failure of fusion of second male gamete with secondary nucleus. In such cases male gamete and secondary nucleus divide independently. It results two kind of tissue in the same endosperm with two different colors. Another view also suggested by Webber that second male gamete fuse with one polar nucleus while second polar nucleus remains haploid. These two structure further divide independently and lead a mosaic endosperm.

3. **Xenia:** It is the effect of pollen (male plant) on seed or fruit (female plant). The term xenia was introduced by Focke in 1881 e.g. maize; some maize grains contain yellow endosperm (dominant) while some white (reccesive).

4. **Metaxenia:** It is the effect of pollen (male plant) on somatic tissue occurred outside of the embryo sac (female plant). The term metaxenia was introduced by Swingle in 1928 e.g. in date palm, the fruit size and maturation time varies with the pollen fertilizing the ovule.

Morphological nature of endosperm

1. In gymnosperm, endosperm is gametophytic tissue because it is derived from megaspore nucleus.

2. In angisperms, its nature is debatable and different theories originated from time to time to explain the morphological nature of endosperms which are as follows:

a) According to Monnier and Miss Sargent: Endosperm is Sporophyte.

b) According to Strasburger (1900) and Coulter & Chamberlain (1911)**:** Endosperm is gametophyte.

c) According to Nemac, Brink and Cooper (1942): Endosperm is an entirely new structure i.e. “Tissue sui generis”.

Chapter-13

EMBRYO DEVELOPMENT OR EMBRYOGENESIS OR EMBRYOGENY

The development of embryo occurs inside wall of embryo sac, so, it is an endosporic development. The first division in zygote is transverse and a basal and an apical/terminal cell are formed. Basal cell further divides by several transverse division and form a thread of cell called suspensor (6-10 celled). The apical cell first divides by longitudinal and then by transverse division to form globular embryo which later becomes heart shaped. This type of development is most common and primitive represented by *Capsella bursa-pastoris* (cruciferae) and is referred to as Crucifer type / Onagrad type. It was first studied by Hanstein (1870). P. Maheshawari (1950) recognized five kinds of embryogeny on the basis of (1) plane of the division of the terminal daughter cell in two celled pro-embryo (2) the contribution of basal and terminal daughter cell in the formation of embryo proper.

1. Apical cell of the two celled pro-embryo divides longitudinally

a) **Onagrad type/Crucifer type:** It was first worked by Hanstein (1870) in *Capsella bursa-pastoris* (Shephered purse). In this type, basal cell play minor or no role in the development of the embryo e.g. Cruciferae, Ranunculaceae, Annonaceae, Onagraceae, Pedaliaceae etc.

b) **Astrad type:** In this, the terminal cell and basal cell both contribute in the development of embryo. Jones (1927) first showed the astrad type of embrogeny in *Lactuca sativa.*

2. The apical cell of the two celled pro-embryo divides transversely

a) **Solanad type:** First observed by Soueges in *Nicotiana* plant**.** Basal cell play minor role. Basal cell divide and forms two or more celled suspensor e.g. Solanaceae, Liliaceae, Companulaceae, Theacaceae etc.

b) **Caryophyllad type:** It was first observed by Soueges in *Sagina procumbans*. Basal cell play no role and suspensor (if present) also derived from apical cell e.g. Caryphyllaceae, Crasulaceae, Fumariaceae, Holoragaceae etc.

c) **Chenopodiad type:** First observed by Soueges in *Chenopodium bonus henricus.* Both basal as well as terminal cell take part in the formation of embryo e.g. Chenopodiaceae, Boroginaceae etc.

Note: In *Anemone rivularis*, two types of embryogeny Solanad and Crucifer type are seen.

Embryogeny in Dicots: In a typical dicot, the zygote elongates and then divides by a transverse wall into two unequal cells. The larger basal cell is called suspensor cell. The other towards the antipodal end is termed as terminal cell or embryo cell. The suspensor cell divides transversely few times to produce a filamentous suspensor of 6-10 cells. The suspensor helps in pushing the embryo in the endosperm. The first cell of the suspensor towards the micropylar end becomes swollen and functions as a haustorium. The haustorium has wall ingrowths similar to transfer cells. The last cell of the suspensor at the end adjacent to the embryo is known as hypophysis. Hypophysis later gives rise to the radicle and root cap. The embryo cell undergoes two vertical divisions (quadrant stage) and one transverse division to form eight cells arranged in two tiers (octant stage), epibasal (terminal) and hypobasal (near the suspensor). The epibasal cells 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 differentiate further into procambium (= plerome) and ground meristem (= periblem). Protoderm forms epidermis, procambium gives rise to stele or vascular strand and ground meristem produces cortex and pith. Initially the embryo is globular and undifferentiated. Early embryo with radial symmetry is called pro-embryo. It is transformed into embryo with the development of radicle, plumule and cotyledons. Two cotyledons differentiate from the sides with a faint plumule in the centre. At this time the embryo becomes heart-shaped.

Structure of dicot embryo: A typical dicot embryo consists of an embryonal axis and two cotyledons. The part of embryonal axis above the level of cotyledons is called epicotyls. It terminates with the stem tip and called plumule (future shoot). The part below the level of cotyledons is called hypocotyls which terminates in the root tip and called radicle (future root). The root tip is covered with a root cap (calyptras). In some plants the embryo remains in the globular or spherical form even at the time of seed shedding without showing and distinction of plumule, radicle, and cotyledons e.g. Orobanche, Orchids, Utricularia etc.

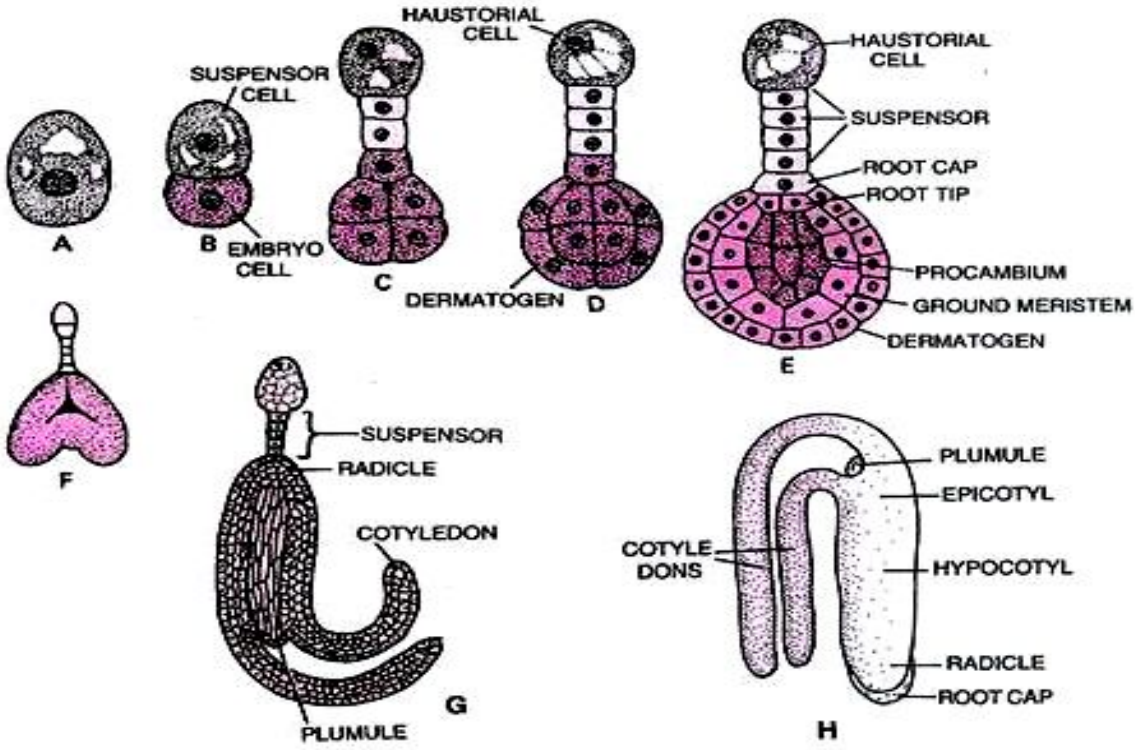


Fig. 13.1, Stages in the development of a dicot embryo. A, Zygote or oospore. B, Division of zygote into suspensor and embryo cells. C, Formation of suspensor and embryo octant. D, Periclinal division of embryo octants to form outer dermatogens. E, Globular embryo showing regions radical, procambium, ground meristem and dermatogens. F, Heart shaped embryo. G, Mature dicot embryo. H, a typical dicot embryo

Embryogeny in Monocots: The zygote or oospore elongates and then divides transversely to form basal and terminal cells. The basal cell (towards micropylar end) produces a large swollen, vesicular suspensor cell. It may function as haustorium. The terminal cell divides by another transverse wall to form two cells. The top cell after a series of divisions forms plumule and a single cotyledon. Cotyledon in this case called scutellum which grows rapidly and pushes the terminal plumule to one side. The plumule comes to lie in a depression. The middle cell, after many division forms hypocotyl and radicle. It also adds a few cells to the suspensor. In some cereals both plumule and radicle get covered by sheaths developed from scutellum called coleoptile and coleorhiza respectively.

Structure of Monocot Embryo: The embryos of monocotyledons have only one cotyledon. In grass family (Gramineae), this cotyledon is called scutellum. It is situated towards lateral side of embryonal axis. This axis at its lower end has radicle and root cap enclosed in a sheath called coleorhiza. The part of axis above the level of attachment of scutellum is called epicotyl. It has a shoot apex and few leaf primordia enclosed in a hollow foliar structure called coleoptile. Epiblast represents rudiments of second cotyledon.

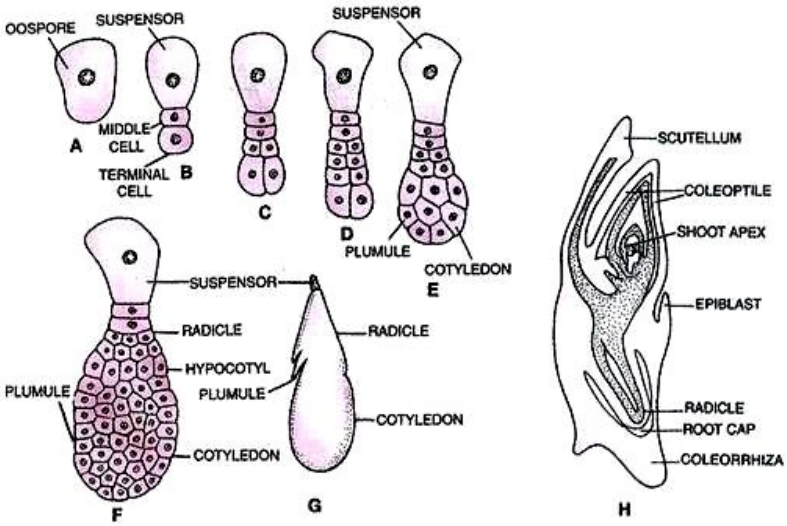


Fig. A-G; Stages in development of monocot embryo, H; a monocot embryo of a grass

Chapter-14

Special Modes of Reproduction

Polyembryony and Apomixis

Polyembryony: Polyembryony is the occurence of more than one embryo inside the seed but finally only one embryo develops into a seedling. Polyembryony was first reported by Leeuwenhoeck (1719) in Citrus. It is more frequently occurs in gymnopsperms than angiosperms because in gymnosperms each ovule produces a number of archegonia which get fertilized independently and give rise to additional embryos. Ernst **(1901)** sub-divided naturally occurring polyembryony into two categories i.e. false and true. In false polyembryony, the embryo develops from separate embryo sac present in same ovule e.g. *Frageria, Bergania.* In case of true polyembryony, an extra embryos arise in the same embryo sac i.e. from nucellus (e.g. *Citrus, Mango, Opuntia*) or from synergids (e.g. *Sagitaria, Argemone*) or from antipodal cells (e.g. *Ulmus Americana*), or from endosperm (e.g. *Balanophora*) or from integuments (e.g. *Limnanthes, Spiranthes australis*). Polyembryony may arise by the following various methods:

Adventive polyembryony: Formation of extra embryos through sporophytic budding is called adventive polyembryony e.g. embryo forms from the nucellus which penetrate the embryo sac e.g. Mango, *Citrus* etc.

Cleavage polyembryony: It is reported by Jaffery (1895) in *Erythronium americana* and it is due to cleavage of zygote. It is more common in gymnosperm *(Pinus)* than angiosperm. In the angiosperm family Orchidaceae e.g. *Eulophea epidendraea*, cleavage polyembryony occurs more frequently.

Induced polyembryony: Each viable cell of plant can be converted into an embryo by providing suitable environmental condition and nutrition in vials. Such embryo is called adventive embryo e.g. haploid embryo from pollen grains, diploid embryo from nucellus or integuments. More than 100 plant species have been taken for successful production of induced embryo e.g. carrot, butter cup, wheat, grapes, citrus, coffee sp. etc. In *Allium odorum* five embryos develop by different methods e.g. 1-zygotic, 1-synergids, 2-antipodals and one from integuments. Maximum number of embryo i.e. 40 is reported inside single seed of *Citrus unshiu.*

Causes of polyembryony: Different theories have been proposed to explain the causes of polyembryoby

1. **Necrohormone theory:** It is the most accepted theory and proposed by Haberlandt (1921). According to him, degenerating nucellar cells release some substances which stimulate adjacent cell to divide and form embryo.
2. **Reccesive gene theory:** According to Leory (1947), it occurs due to occurrence of one or more recessive gene.

Practical importance of polyembryony: In horticultural plants (e.g. *Citrus,* Mango) genetically uniform parental type seedlings are obtained from nucellar embryo because nucellar embryo seedlings are disease free and maintain superiority for a long duration. (Superior over obtained by cutting /vegetative propagation).

Apomixis: The plants where the usual sexual reproduction (amphimixis) has been completely replaced by a type of asexual reproduction called apomictic and the phenomenon is called apomixis. The term apomixis was coined by Winkler (1908). It may be defined as abnormal kind of sexual reproduction in which egg cells or other cells associated with egg (synergids, antipodals) develop into embryo without fertilization and with or without meiosis. (Apomixis is unusual sexual reproduction where there is no meiosis and syngamy). There are two main categories of apomixis:

A. **Vegetative reproduction:** In this type of apomixes, the new individual arises from a group of undifferentiated or differentiated cells, where neither embryo nor seed are produced. In other words, Plant develops from the cells other than seeds e.g. bulbs, bulbils, suckers, tubers etc.

B. **Agamospermy:** In this type of apomixes, seeds are produced and embryo formed but meiosis and fertilization are eliminated. It is of the following types:

1. **Adventive embryony:** Development of embryo directly from the diploid sporophytic tissue like nucellus and integuments e.g. *Citrus*, *Mango* etc. No alternation of generation because diploid cells of sporophyte directly gives rise to new embryo.

2. **Diplospory:** It is the change of megaspore mother cells directly into embryo sac. In it archesporium develop inside the nucellus of ovule which give rise to mega spore mother cell which directly without meiosis develops into embryo sac (having diploid egg, synergids, and antipodal cells) e.g. *Taraxacum*, *Aerva tomentosa.*

3. **Apospory:** It was discovered by Rosenberg in angiosperms. It is the change of sporophyte into embryo sac. In this, somatic cells in nucellus or integument directly forms embryo sac. In case of **somatic apospory,** diploid nucellus cells or integumetic cells enlarge and its nucleus undergoes three free nuclear divisions forming diploid embryo sac. Such embryo sac is called aposporatic embryo sac and the phenomenon is called somatic apospory e.g. *Ranunculus, Crepis, Hypericum, Mallus* etc. Embryo may develop from any diploid cell of the embryo sac. In **generative apospory**, embryo sac arises from the diploid primary archesporial cell e.g. *Parthenium argentatum* etc.

4. **Apogamy:** It is the change of gametophyte other than egg into sporophyte. Sometimes the embryo is formed from the synergid or antipodal cell (except egg) without fusing with the male gamete. This phenomenon is called apogamy. It is of two types. In diploid apogamy there is no reduction in the number of chromosomes of mega spore mother cells, consequently all the cells of embryo sac become diploid. Any diploid cells like synergid, antipodal cells develop directly into embryo is known as diploid apogamy e.g. *Allium* etc. In case of haploid apogamy, reduction in megaspore mother cells occur which develops consequently into embryo sac having haploid cells. Any haploid cell like synergid, antipodal if develop into embryo is called haploid apogamy e.g. *Lilium*

5. **Parthenogenesis:** It is development of zygote from the egg cell without the act of fertilization or in other words it can be said as that the formation of embryo from an unfertilized egg is called parthenogenesis. It is of the following two types: **In diploid parthenogenesis**, there is no reduction in the number of chromosomes in megaspore mother cell which producing diploid 8 nucleate embryo sac. Consequently all the eight nuclei of the embryo sac are diploid and so the egg nuclei. Hence diploid egg give rise to an embryo without fertilization is called diploid parthenogenesis. In haploid parthenogenesis, megaspore mother cell undergoes reduction division. The haploid megaspore producing embryo sac with haploid cells including egg. Haploid egg if somehow give rise to embryo without the act of fertilization is called haploid parthenogensis. It occurs rarely and has been reported in *Solanum nigrum.*

According to famous embryologist P. Maheshwari, there are two types of apomixes:

A. **Recurrent apomixes:** Vegetative propagation and agamospermy are included in recurrent apomixes in which diploid embryo developed. All the nuclei of embryo sac are diploid and there is no meiotic division. Embryo arises either from egg (parthenogenesis) or from other cell of the gametophyte (diploid apogamy).

B. **Non-Recurrent apomixes:** Megaspore mother cell (2n) after normal meiosis produces megaspore (n) and then megaspore develops into haploid embryo sac (n). If haploid egg from haploid embryo sac without fertilization develops into embryo (n), it is known as haploid parthenogenesis. If other haploid cells in the embryo sac other than egg develop into embryo (n) without fertilization, it is known as haploid apogamy.

Parthenocarpy: The term parthenocarpy was given by F. Noll (1902). It is the development of ovary into fruit without pollination and fertilization. Parthenocarpic fruits are either seedless or contain abortive, empty or non-viable seeds. Parthenocarpy is of common occurrence in the nature in the

varieties of banana, cucumber, orange, pine apple, grapes etc. Parthenocarpy can be induced artificially by spraying growth promoting substances such as naphthalene acetic acid (NAA), a type of auxin. This is called induced parthenocarpy. Parthenocarpy induced by various means such as:

1. By spraying auxins, especially NAA (applied after anthesis) and GA (applied at anthesis).
2. By delaying pollination.
3. By using foreign pollen grains.
4. By use of powdered pollens and pollen extracts.
5. By cutting style from base and applying chemicals like IAA, IBA etc. in lanolin paste at cut surface.

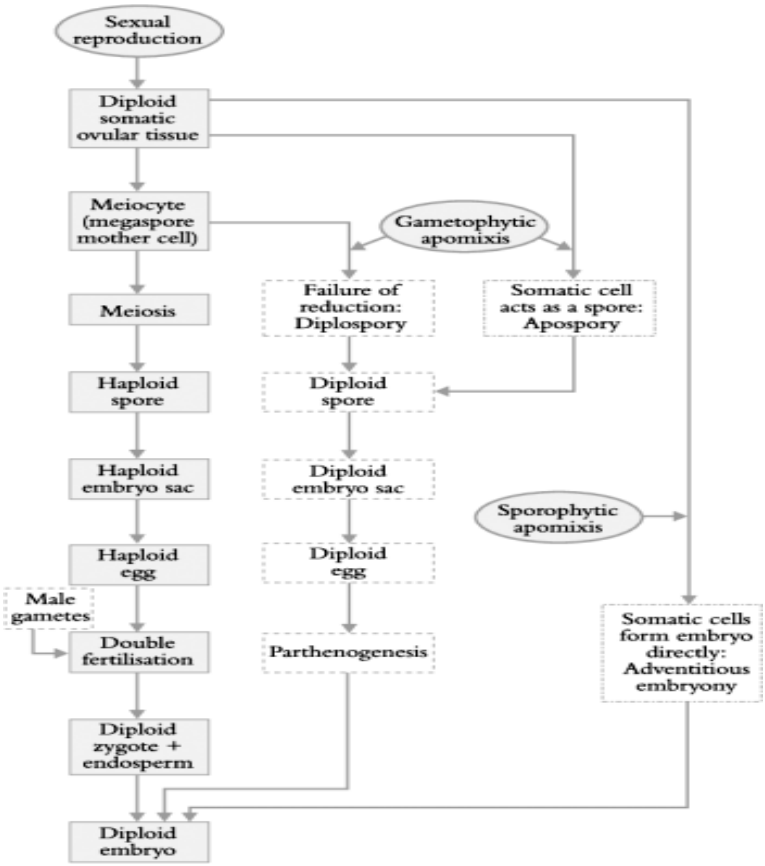


Fig. 14.1, A schematic representation of sexual reproduction and apomixis

Chapter- 15

Alternation of Generation

As in other group of plants, life cycle of angiospermous plants too shows two distinct generations to complete the life cycle. One of these generations is called haploid or gametophytic generation and the other is called diploid or sporophytic generation. These two generations alternate with each other to complete the life cycle. This phenomenon is called alternation of generation. Concept of alternation of generation was given by Strassburger.

Sporophytic phase: It is diploid (2n) phase. In angiosperms, the main plant body belongs to this phase which differentiates into root, stem and leaves. It is dominant stage and it is developed from diploid zygote. Reproductive organs (flowers) developed on this plant. Flowers bear stamens (microspotrophylls) and carpels (megasporophylls). Stamens bear four microsporangia which produce micropsore mother cells. Each micropsore mother cell undergoes meiotic division producing four haploid micropsore. Microspore on germination forms the mature male gametophyte whci is highly reduced and consists of two male gametes, tube nucleus and the pollen tube. Each carpels may have one or more ovules (megasporangia). In the nucellus of ovule, megaspore mother cell developed and producing four megaspores by meiosis. Out of these four, only one remains functional giving rise to eight nucleated embryo sac (female gametophyte). With the formation of microspore (haploid) and megaspore (haploid) gametophyte stage begins. Female gametophyte (embryo sac) contains egg and male gametophyte contains two male gametes.

Gametophytic phase: It is haploid (n) phase. It is developed from haploid microspores and megaspores which are products of meiotic divisions of diploid microspore and megaspore mother cells respectively. Consequently the microspore and megaspores germinate and form the male and female gametophytes respectively. The male gametophyte gives rise to two male gamete and female gametophyte forms the female gamete (egg cell). A diploid zygote is formed by the fusion of one male gamete and egg. Other male gamete fuses with secondary nucleus (n+n) to form triploid endosperm nucleus which give rise to the endosperm. The zygote undergoes repeated mitotic divisions and later become an embryo in the seed.

With formation of diploid zygote, sporophytic phase again begins. In this way alternation of generation between sporophyte phase and gametophyte phase going on to complete the life cycle. It is to be remembering that zygote is the first cell of sporophytic phase while the spore mother cell is the last cell. Spore (haploid) is the first cell of gametophyte while the gametes (sperm and egg) are the last cell.

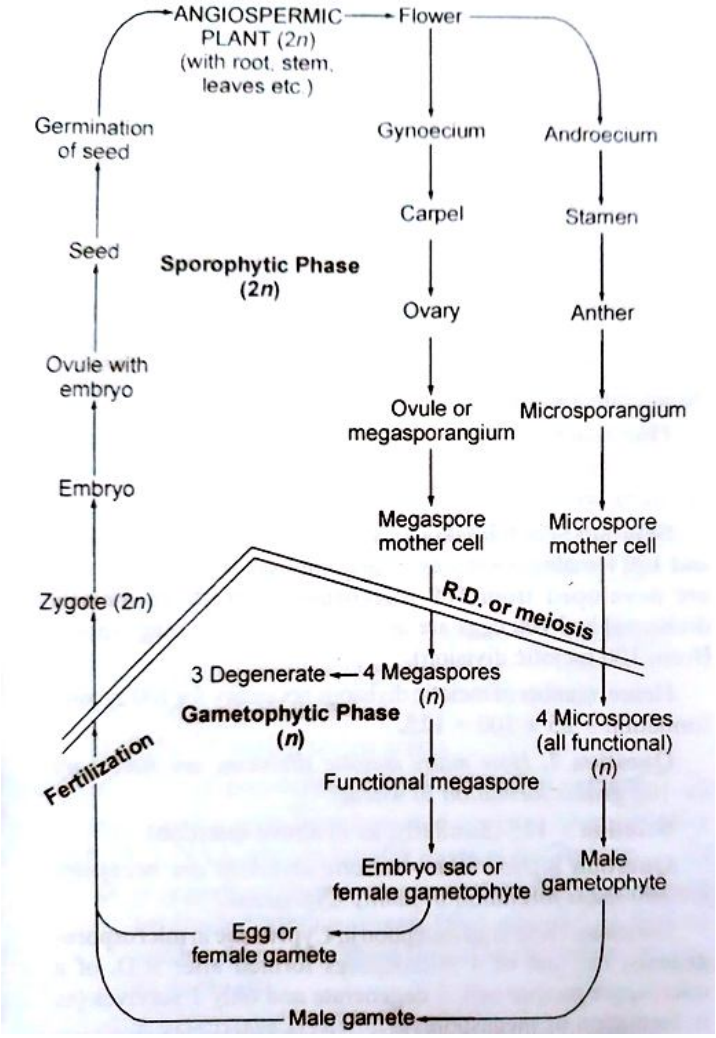


Fig. 15.1, Graphic representation of alternation of generation in a typical angiospermic plant

Salient features of angiosperms

1. The angiosperms comprises of highest number of plants almost adapted to all types of environment and habitats.
2. The main plant body is sporophyte generally differentiated into root, stem and leaves.
3. They are of various habits like herbs, shrubs, trees, climbers.
4. They show various modes of nutrition like epiphytic, parasitic, symbiotic etc.
5. Well developed vascular systems are present.
6. Secondary growth is common in dicot group.
7. Reproductive structure flower is bisexual (perfect flowers) or unisexual (imperfect flowers).
8. Unisexual flowers have carpels (megasporophylls) or stamens (microsporophylls).
9. Bisexual flowers possess both carpels as well as stamens.
10. Carpel is the unit of gynoecium differentiated into stigma, style and ovary. Ovary is reproductive part which encloses ovule. Fertilized ovule matures into seed enclosed within the fruit (fertilized ovary).

11. Stamen is the unit of androecium differentiated into filament and anther (reproductive part). Anther producing microspore (pollens).
12. The plants are heterosporous i.e. microspore and megaspore are produced in the microsporangia and megasporangia respectively.
13. Pollination occurs and microspores are pollinated by the agencies like wind, water, insects, birds etc. Male gametophyte is highly reduced and is represented by germinated microspore having two male nuclei and tube nucleus.
14. Megapsore remains inside megasporangium and gametophyte dependent on the sporophyte. Female gametophyte is represented by 8-nucleated embryo sac.
15. Sexual reproduction is of oogamous type.
16. Double fertilization is common occurrence in angiosperms in which second male gamete fuses with polar nuclei to form endosperm. This is known as triple fusion.
17. The seeds are enclosed inside the fruits.
18. The embryo consists of two lateral cotyledons as in case of dicots while one terminal cotyledon in case of monocots.
19. Seeds may be endospermic, non-endospermic or perispermic.

Chapter-16

Plant Tissue Culture

Plant tissue culture and genetic engineering are the two most widely used methods for crop improvement in plant breeding. Plant tissue culture is the technique of maintaining and growing plant cells, tissues or organs on artificial medium in suitable containers under controlled environmental conditions which is based on the totipotent nature of plant cells or the phenomenon of totipotency. It is one of the latest and most promising methods of crop improvement in those plants where all other conventional methods of breeding fail. The concept of totipotency was given by Gottleib Haberlandt (a German Botanist) in 1902, who cultured fully differentiated plant cells isolated from different plants. He developed the concept of in-vitro culture of plant cells and regarded as the father of tissue culture. With the identification of a variety of chemicals like auxin, cytokinin, other hormones, vitamins, etc. and their role in affecting cell division and differentiation, the methods of plant tissue culture developed in a proper manner. Later on, a number of suitable culture media were developed for culturing plant cells, tissues, protoplasts, embryos, anthers, root tips etc. The cultures are usually kept in culture room at about 24 C^o with some light. Other compounds like casein hydrolase, coconut milk, malt extract, yeast extract may be added for some specific purposes. An optimum pH (usually 5.7) is also very important. The extensively used nutrient medium is Murashige and Skoog medium (MS medium) which was developed by Murashige and Skoog in 1962. The practical application of totipotency was shown by steward in 1932 when he developed a complete carrot plant from a single cell obtained from the root of wild carrot. Foundation of commercial plant tissue culture was laid in 1960 with the discovery for a million fold increase in the multiplication of Cymbidium (an orchid) which was accomplished by G. M. Morel. In India, the work on tissue culture was initiated during 1950s at University of Delhi which is credited to Shri Panchanan Maheshwari (P. Maheshwari) who was working there in the Department of Botany. The land mark in the development of in-vitro culturing of plants was the discovery of haploid production. Shri P. Maheshwari and Sipra Guha made a remarkable contribution in the development of plant tissue culture in India.

Table-16.1, Some of the early contributions in the field of plant tissue culture are tabulated below:

Year	Worker	Advancement
1902	Haberlandt	First attempt of in-vitro culture of plant cell
1904	Hanning	Culture of embryogenic tissue of crucifers
1922	Robbins	In-vitro culture of roots
1925	Laibach	Zygotic embryo culture in *Linum*
1934	White	Culture of roots of tomato plants
1941	Braun	Culture Crown Gall Tissues
1955	Miller	Hormone kinetin discovered
1957	Skoog, Miller	Discovered that auxin : cytokinin ratio regulates the organ formation
1960	Bergmann	Development of plating technique for isolation of single cell
1970	Power	Successful protoplast fusion
1970	Maheshwari and Guha	Successful anther culture
1978	Melchers	Production of somatic hybrid pomato

Tissue Culture: The in-vitro culture of the tissue e.g. Callus culture

Cell Culture: Denotes the in-vitro culture of single or a few cells.

Organ Culture: This term is used for in-vitro culturing of organs like embryo, root or shoot apices.

Suspension Culture: Defined as the culture of cell and cell aggregates suspended in a liquid medium containing auxin (2, 4-D). Suspension culture grows much faster than callus culture. The development

of an organized structure like root, shoot or somatic embryo from cultured cells can be described by regeneration.

Sub-culture: Cells and tissues are regularly transferred into new culture vessels containing fresh media. This process is called sub-culturing.

Ex-plant: A single cell or a group of cells is taken for culture is called as explants e.g. embryos, young leaf, bud, etc. by culturing ex-plant in culture medium; a callus is obtained which is used for organogenesis by the action of hormones.

Callus: The undifferentiated mass of cells is referred to as callus. The cells of callus are meristematic in nature. The unique feature of callus is that the abnormal growth of it has biological potential to develop normal root, shoot and emryoids which ultimately forming a plant. Callus is formed through three developmental stages – inductions, cell division and differentiation within two to three weeks. The young seedlings are transferred to the field for further growth.

Plant tissue culture is fundamental to most aspects of biotechnology of plants. It is evident now that plant biotechnology is one of the most beneficial of all the sciences. The products of plant biotechnology are being transferred rapidly from laboratories to the fields. Also, the plant tissue culture has become of great interest to the molecular biologists, plant breeders and even to the industrialists, as it helps in improving the plants of economic importance. In addition to all these, the tissue culture contributes immensely for understanding the patterns and responsible factors of growth, metabolism, morphogenesis and differentiation of plants.

Basic technique of plant tissue culture:
The basic technique of plant tissue culture includes the followings as:

1. Preparation of suitable nutrient medium
2. Selection of ex-plants
3. Sterilization ex-plant with suitable disinfectants and then washing it with sterile distilled water.
4. In-oculation of ex-plants into suitable nutrient medium.
5. Incubation i.e. growing the culture in growth chamber having suitable light, temperature and relative humidity.
6. Regeneration of plants from cultured plant tissues.
7. Hardening (gradual exposure to environmental condition).
8. Plantlets transferred to green house or field conditions.
9. Explants are treated with specific antimicrobial chemicals to make them free from microbes. The vessels, media and instruments are also suitably treated with steam, dry heat or alcohol or subjected to filtration to make them free from microbes and this called surface sterilization.
10. Culture medium provides the nutrition that is required for the desired growth and development of the explants.
11. Standard media are available which contain inorganic salts, some vitamins, sucrose and desired growth regulators.

Important constituents of a culture medium

1. **Organic supplements:** Vitamins like thiamine (B_1), Pyridoxin (B_6), Nicotinic Acid (B_3), etc. antibiotics like Streptomycin; amino acid like Arginine, Asparagine.
2. **Inorganic Nutrients:** Micronutrients as Iron (Fe), Manganese (Mn), Zinc (Zn), Molybdenum (Mo), Copper (Cu), Boron (B); Macronutrients include six major elements as Nitrogen (N), Sulphur (S), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg).

3. **Carbon and Energy Source:** Most preferred carbon source is sucrose. Others include lactose, maltose, galactose, raffinose, cellobiose, etc.
4. **Growth Hormones:** Auxins-mainly for inducing cell division; cytokinins-mainly for modifying apical dominance and shoot differentiation; Abscisic Acid (ABA)-Used occasionally; Gibberellins-Used occasionally.
5. **Gelling Agents:** These are added to media to make them semisolid or solid. Agar, Gelatin, Alginate etc. are common solidifying or gelling agents.
6. **Other Organic Extracts:** Sometimes culture media are supplemented with some organic extracts also like coconut milk, orange juice, tomato juice, potato extract, etc.

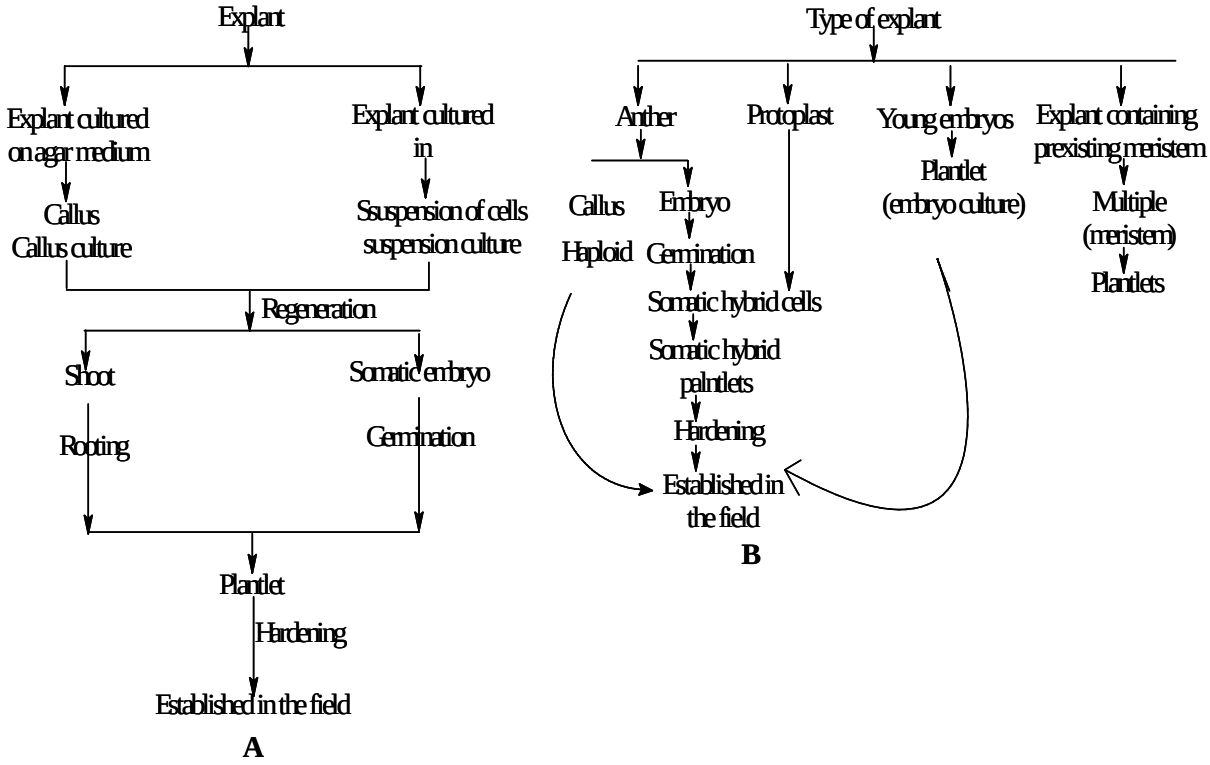


Fig. 16.1, A flow chart showing the various types of plant tissue culture and recovery of complete plant from them

A. Callus and suspension culture, B. Anther, Protoplast, Embryo and Shoot meristem culture

Types of plant tissue culture: Plant tissue cultures are classified according to the type of in-vitro growth viz. callus and suspension:

Callus Culture: In callus culture, cell division in explants forms a callus. Darkness and solid medium gelled by agar stimulates callus formation. The medium generally contains the auxin like 2, 4-D and often a cytokinin like BAP. This stimulates the cell division in explants. Callus is obtained within 2-3 weeks.

Suspension Culture: A suspension culture consists of single cells and small groups of cells suspended in a liquid medium containing auxin like 2, 4-D. Suspension culture grows much faster than callus culture. Suspension culture must be constantly agitated at 100-250 rpm which serves the purposes like (a) aeration of culture (b) constant mixing of medium (c) breakage of cell aggregates into smaller cell groups.

The callus and suspension cultures may be used to achieve cell biomass production, regeneration of plantlets, and production of transgenic plants and isolation of protoplasts.

Embryo Culture: Embryo culture is the technique of taking out young embryos from developing seed and their growth on culture medium to form seedling and then young plants. They have number of applications as follows:

1. **Embryo Rescue:** it is taking out the fragile embryos from fertilized ovules of interspecific crosses before their abortion and culturing them to form viable hybrid seedlings e.g. Jute, Tomato, bean etc.
2. **Orchid:** Orchid seeds lack store food. Embryo culture helps in developing from all the seeds. The technique is also used in clonal multiplication.
3. **Dormant Seeds:** Inhibitors present in endosperm and other parts of seeds do not allow the embryos to grow. Embryos of such seeds can be excised and grow over culture medium to form seedlings. It eliminates the action of inhibitors and dormancy.

Anther Culture and Haploid Production: When anthers of some plants are cultured on a suitable medium to produce haploid plants, it is called anther culture. The most important method of haploid production is pollen culture. Haploid production through pollen culture was first made in *Datura innoxia* (Jimson weed) by Guha and Maheshwari. Haploids are completely sterile and of no direct value but they are very important in plant breeding because –

i. They have single set of chromosomes, so even a very small change or mutation can be detected in haploids.

ii. They are used to produce homozygous diploids (by colchicine treatment) and these homozygous diplods are used as parents in crossing programme.

Ovule Culture: Culturing of fertilized ovules is sometimes needed in those cases where embryos abort very early and the culture of embryo is not possible due to difficulty in excision at a very early stage. Ovules can easily be excised from the ovary and cultured on the usual basal medium. In most of the cases, ovaries are often cultured either for in-vitro pollination, fertilization or embryo rescue. In cases of interspecific or intergenic crosses, the ovaries are excised at the zygote stage or at the two-celled pro-embryo stage and cultured on synthetic media. Fruits can be successfully obtained by culturing ovaries on synthetic media containing coconut milk, auxin or any other specific requirement.

Protoplast Culture or Somatic Hybridization: The process of producing somatic hybrids (fusion of cells of two varieties or species) by PEG (Polyethyelene glycol) is called somatic hybridization. The plant cell without cell wall is called protoplasts. The first step in somatic hybridization is the removal of cell wall by digestion with a combination of pectinase and cellulase. The naked protoplasts are fused by electro-fusion (high frequency alternating electric field with short current phases) or chemo-fusion (through sodium nitrate or PEG=polyethylene glycol). It results in hybrid protoplasts. The somatic hybrid may have syn-karyon (single fused nucleus) or hetero-karyon (having two un-fused nuclei). Somatic hybrids may be used for the production of useful allopolyploids. Somatic hybrids in plants were first obtained between two species of tobacco (*Nicotiana glauca* and *N. langsdorffii*) by Carlson et.al. in 1972. Successful somatic hybrids have also been got different species of *Brassica, Putunia* and *Solanum*. Pomato is a somatic hybrid between tomato and potato and is an intergenic hybrid. Somatic hybrids are also produced between rice and carrot. Protoplast technology has opened up avenues for dvelopemnt of hybrids of even asexually reproducing plants. Genetic manipulation can be carried out more rapidly when the plant cell is in protoplast state. New genes can be introduced (e.g. male sterility, herbicides resistance) easily in this state and mutations will be also easier.

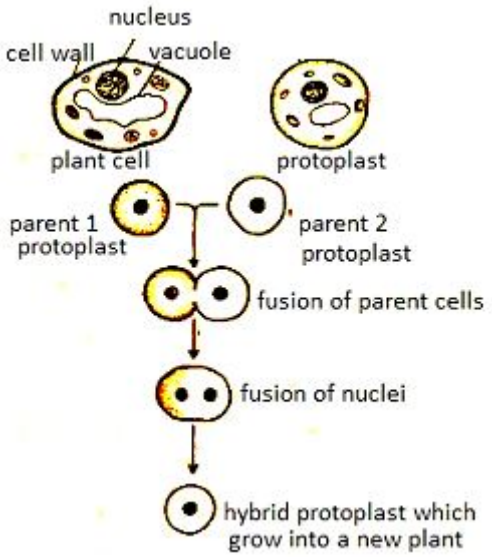


Fig. 16.2, A diagrammatic representation of protoplast fusion

Meristem Culture or Micropropagation: When an explant that contains pre-existing shoots meristems and produce shoots from them. Such cultures are called meristem culture. Because of the minute size of the propagule in the culture, the propagation technique is known as micropropagation. The explants generally used in meristem culture are shoot tips and more often nodal segments (axillary meristems). These explants are cultured on a medium containing a cytokinin (usually BAP). Cytokinin promotes axillary branching by overcoming apical dominance. Therefore, they support multiple shoot development from each explant. When axillary branching takes place, individual shoots are cultured but when axillary branching does not takes place, the single shoot is cut into nodal segments which are then cultured again. Shoots of 2-3 cm are excised and rooted on a suitable medium. The plantlets thus obtained are subjected to hardening and finally established in the field. Scientists have succeeded in culturing meristems of banana, sugarcane, potato etc.

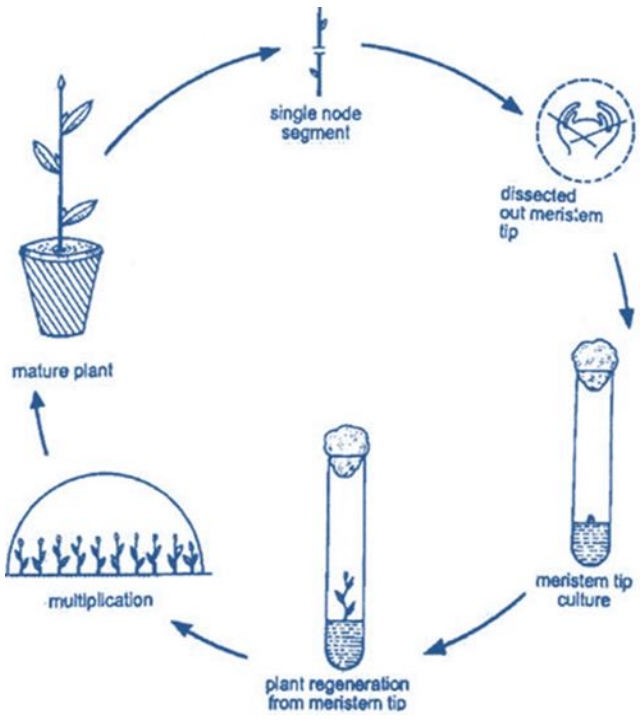


Fig. 16.3, Regeneration of plants through meristem culture

Advantage of Meristem Culture:

1. It helps in rapid multiplication of plants; it is an easy, safe and economical method for plant propagation.
2. It is used for virus free production of plants.
3. Sterile plants are multiplied by this method.
4. It is used in commercial production of ornamental plants like lilly, orchids, Eucalyptus, Chinchona, tomato, apple, banana, grapes, citrus etc.
5. Generally similar plants are produced by this method; therefore, desirable characters and desired sex of superior variety are kept constant for many generations.
6. The rare plant and endangered species are multiplied by this method and such plants are saved.

Application of Plant Tissue Culture

All the cells in callus or suspension culture are derived from single explants by mitotic division. Therefore all the plantlets obtained in this way possess the same genotype and constitute a clone. These plantlets are used for rapid clonal propagation. Genetic variation present among plant cells of a culture is called somaclonal variation.

Weedicides are added to culture initially in very small concentration and dosage increased in subsequent cultures till the desired level of resistance is obtained. The resistant cells are then regenerated to form plantlets and plants

1. Pre-existing shoot meristem can be used as explants to produce shoots. Such cultures are known as meristem culture. It is used in production of disease free plants. Cytokinin is the culture medium in meristem culture e.g. banana, cardamom, etc. are produced by this method.
2. When anthers of some plants are cultured on a suitable medium to produce haploid plants, it is called anther culture. The most important method of haploid production is pollen culture. Haploid production through pollen culture was first made in *Datura innoxia* (Jimson weed) by Guha and Maheshwari. Haploids are very important in plant breeding because:

i. They have single set of chromosomes, so even a very small change or mutation can be detected in haploids.

ii. They are used to produce homozygous diploids (by colchicine treatment) and these homozygous diplods are used as parents in crossing programme.

3. **Somatic hybridization:** The process of producing somatic hybrids (fusion of cells of two varieties or species) by PEG (polyethylene glycol) is called somatic hybridization. The separation of protoplast is done by treating cells with pectinase and cellulase enzymes. The best example of somatic hybrid is Pomato which is the somatic hybrid of tomato and potato and is an intergenic hybrid.
4. **Micropropagation** is the use of plant tissue culture to regenerate large number of plants. This results in production of genetically identical plants and it is also called as clonal propagation. Micropropagation is widely used in forestry and floriculture. It can also be used to conserve rare and endangered plant species.
5. Parental variation present among plant cells of a culture is called somaclonal variations.
6. The entire vegetatively produced descendents of somatic cells are collectively called as clone. An individual member of a clone is called ramet.

Table-16.2, Advantage and disadvantage of tissue culture

S. No.	Advantages	Disadvantages
1.	A lot of new plants can be grown in relatively short time	All plants have same genetic make-up, so they will be vulnerable to same

		diseases or pests.
2.	Little space is needed.	No chance of new beneficial characteristics arising by chance.
3.	All new plants inherit the same desirable characteristics.	No variation means there is the danger of reducing the gene pool.

Table-16.3, Difference between callus and suspension culture

S. No.	Callus culture	Suspension culture
1.	In this culture, cell division in explants forms callus, Callus is an irregular, unorganized and undifferentiated mass of actively dividing cells.	It consists of single cells and small group of cells suspended in a liquid medium
2.	The culture is maintained on agar medium.	The culture is maintained in liquid medium.
3.	The medium contains growth regulators, the auxin such as 2, 4-D and cytokinin like BAP.	The medium contains growth regulator, the auxin usually 2, 4-D only.
4.	Callus is obtained within 2-3 weeks.	Suspension culture grows much faster than that of callus culture.
5.	It does not need to be agitated	It must be constantly agitated at 100-250 rpm.

Some basic steps in tissue culture

Selection and Sterilisation of Explant: Suitable explant is selected and is then excised from the donor plant. Explants are then sterilized using disinfectants.

Preparation and Sterilisation of Culture Medium: A suitable culture medium is prepared with special attention towards the objectives of culture and type of explant to be cultured. Prepared culture medium is transferred into sterilized vessels and then sterilized in autoclave.

Inoculation: Sterilized explant is inoculated (transferred) on the culture medium under aseptic conditions.

Incubation: Cultures are then incubated in the culture room where appropriate conditions of light, temperature and humidity are provided for successful culturing.

Sub culturing: Cultured cells are transferred to a fresh nutrient medium to obtain the plantlets.

Transfer of Plantlets: After the hardening process (i.e., acclimatization of plantlet to the environment), the plantlets are transferred to green house or in pots.

Cellular Totipotency

The potential of a plant cell to grow and develop into a whole new multicellular plant is described as cellular totipotency. In other words, the property of a single cell for differentiating into many other cell types is called as totipotency. This is the property which is found only in living plant cells and not in animal cells (exception being stem cells in animals). The term totipotency was coined in 1901 by Morgan. During culture practice, an explant is taken from a differentiated, mature tissue. It means, the cells in explants are generally non-dividing and quiescent in nature.

To show totipotency, such mature, non-dividing cells undergo changes which reverts them into a meristematic state (usually a callus state). This phenomenon of reverting back of mature cells to dividing state is called dedifferentiation. Now, these dedifferentiated cells have the ability to form a

whole plant or plant organ. This phenomenon is termed as re-differentiation. Dedifferentiation and re-differentiation are the two inherent phenomena involved in the cellular totipotency. Regarding this, it is clear that the cell differentiation is the basic event for development of plants and it is also referred to as cyto-differentiation. To express its totipotency, a differentiated cell first undergoes the phenomenon of dedifferentiation and then undergoes the re-differentiation phenomenon (Fig. 3). Usually the dedifferentiation of the explant leads to the formation of a callus. However, the embryonic explants, sometimes, result in the differentiation of roots or shoots without an intermediary callus state.

Thus, from the above account it is clear that unlike animals (in which differentiation is irreversible usually), the plants have such a quality that even highly mature and differentiated cells have an ability to revert back to meristematic state. The property of totipotency of plant cells indicate that even the undifferentiated cells of a callus carry the essential genetic information required for regeneration of a whole plant.

It is also clear that all the genes responsible for dedifferentiation or re-differentiation are present within the individual cells and they become active for expression under adequate culture conditions. As totipotent cells are the basis of whole plant tissue culture techniques, so, by the exploitation of this potential of plant cells, biotechnologists are trying to improve the crop plants and other commercially important plants.

Totipotency in Different Plant Parts

The somatic cells in plant body are totipotent. It is to be noted here that only the living plant cells have the ability to regenerate and the dead cells which lack cytoplasm and nucleus (tracheid's, vessel elements, etc.) are not totipotent at all. Different plant parts have different totipotent abilities. For example, in tobacco plant, the type of bud formed by in-vitro culture of the epidermis of different regions of the plant is different in their form. Another example to add here may be given about the totipotency of crown-gall cells which have the capacity to grow as an un-organised mass of cells under normal conditions, however whole plants can be recovered from them in culture. Thus, it is clear that totipotency is not similar in all plant parts.

Applications of Totipotency

Cellular totipotency of plants cells has proved to be a boon to mankind as it is the basis of plant tissue culture. The plant tissue culture exploits this unique property of plant-cells to attain commercial benefits. Various applications of cellular totipotency are:

1. It has potential applications in the crop plant improvement.
2. Micro-propagation of commercially important plants.
3. Production of artificial or synthetic seeds.
4. It helps in conservation of germplasm (conservation of genetic resources).
5. This ability is utilized for haploid productions.
6. Applied in producing somatic hybrids and cybrids.
7. Helps in cultivation of those plants whose seeds are very minute and difficult to germinate.
8. Also helps to study the cytological and histological differentiations.
9. For high scale and efficient production of secondary metabolites.
10. The genotypic modifications can also be possible.

Differentiation: While studying totipotency, it is stated that the dedifferentiation and redifferentiation processes result in the differentiated plant organs, finally producing a whole plant. In case of plants, the differentiation is reversible but in animals, it is irreversible.

The term differentiation describes the development of different cell types as well as the development of organized structures like roots, shoots, buds, etc., from cultured cells or tissue.

Differentiation may also be defined in simple words as the development change of a cell which leads to its performance of specialized function. However, normally morphological characteristics. For example, differentiation accounts for the origin of different types of cells, tissues and organs during the formation of a complete multicellular organism (or an organ) from a single-celled zygote.

Actually, the development of an adult organism starting from a single cell occurs as a result of the combined functioning of cell division and cell differentiation. Various techniques of tissue culture provide not only a scope of studying the factors governing totipotency of cells but also serves for the investigation of patterns and factors controlling the differentiation.

Types of Differentiation:
As stated earlier also, the plant cells have a tendency to remain in a quiescent stage which may be reverted to the meristematic stage. This process is termed as dedifferentiation and as a result of this, a homogeneous undifferentiated mass of tissue i.e., callus is formed. There callus cells then differentiate into different types of cells or an organ or an embryo. **On this basis, the differentiation may be of the following types:**

a) Cyto-differentiation

b) Organ Differentiation

c) Embryo Genic Differentiation

Cyto-differentiation: The differentiation of the cells is an important event of the development of plants. The differentiation of different types of cells from the cultured cells is known as cytodifferentiation. When an undifferentiated callus re-differentiates into whole plant, it first undergoes cytodifferentiation. Amongst different cytodifferentiations, the differentiation into vascular tissues has received maximum attention. However, it is important here to mention that the cells of mature xylem elements and phloem cells cannot be re-differentiated or cannot be reverted back to the meristematic state due to lack of cytoplasm in them. Although, in initial stages of their development, they can be reverted to meristematic cells. Xylogenesis is the differentiation of parenchymatous cells (of callus) into xylem-like cells of vascular plants. Phloem differentiation is the formation of phloem-cells from parenchyma in culture.

Factors affecting cytodifferentiation
(i) Physical factors like light, temperature and pH are effective at optimum levels.

(ii) Chemical factors.

a) Low nitrogen content increases vascularization

b) High Ca^{++} ions stimulate the formation of tracheid's and sieve tubes.

c) Sucrose in high concentration results in pronounced xylem differentiation.

(iii) Hormones
Some hormones play important role in cytodifferentiation. **These are:**

i. Auxin plays major role in vascularization.

ii. Cytokinin promotes cytodifferentiation.

iii. Gibberellins along with auxins promote it.

iv. Abscisic acid inhibits it usually.

Organ Differentiation
It is synonymous to organogenesis or organogenic differentiation. It refers to the development or regeneration of a complete organised structure (or whole plant) from the cultured cells/tissues (Fig. 4).

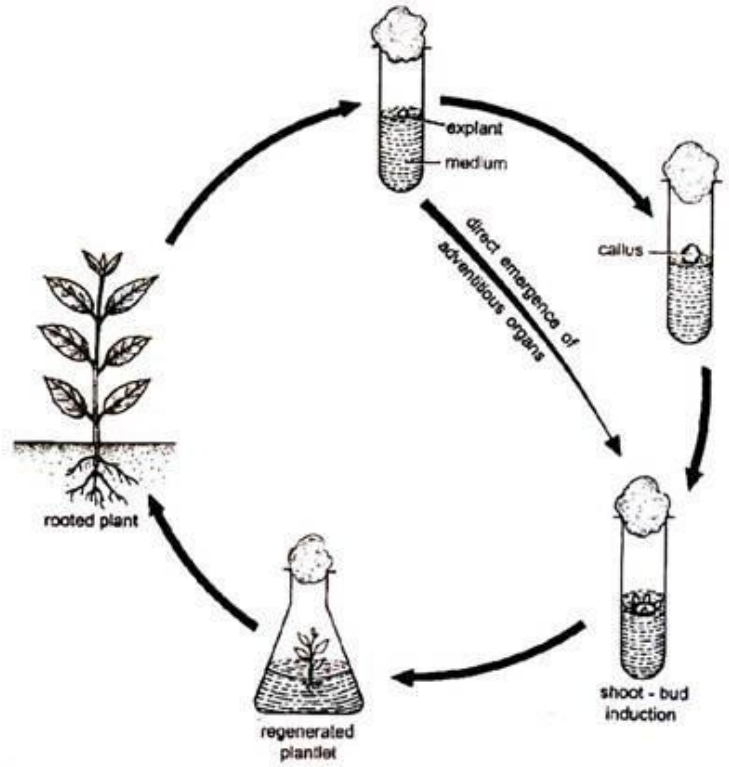


Fig. 16.5, Organogenic Differentiation

ABOUT AUTHORS

Dr. Azahar Sajjad has completed all his educational qualification from Aligarh Muslim University. He has twelve years of teaching and research experience. He has also published more than 20 research papers in journal of national and international repute.

Dr. Akil Ahmad Khan has completed all his educational qualification and research work from Aligarh Muslim University and has vast research and teaching experiences in plant Virology. He has published more than thirty research papers in journals of national and international fame. He also participated in different seminars and symposia.

Empyreal Publishing House
India | UAE | Nigeria | Uzbekistan | Montenegro
ISBN 978-81-946373-7-0