LABORATORY MANUAL

FIRST DEGREE PROGRAMME (CBCSS)
IN BOTANY
UNIVERSITY OF KERALA

VOLUME I

POST GRADUATE AND RESEARCH DEPARTMENT OF BOTANY
SREE NARAYANA COLLEGE, KOLLAM

LABORATORY MANUAL

FIRST DEGREE PROGRAMME (CBCSS) IN BOTANY
UNIVERSITY OF KERALA
VOLUME I

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Laboratory Manual

First Degree Programme in Botany (CBCSS) University of Kerala

Volume I

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PREFACE

This book entitled Laboratory Manual First Degree Programme in Botany (CBCSS) University of Kerala Volume I is prepared as a user-friendly reference material for the practical work of Under Graduate students in Botany as per the Syllabus of the University of Kerala. The book covers the topics in the first Semester and second Semester of First Degree Programme in Botany under Choice based Credit and Semester System. We tried to incorporate as much details as possible in the illustrations made in the Manual. The notes to each illustration needed for the practical examination are included. Hope this Laboratory Manual would be of great help to the students and teachers in their practicals.

All suggestions to improve the contents in the book are gratefully accepted.

Authors

Kollam

11/10/2022

ACKNOWLEDGEMENT

We gratefully acknowledge the Manager, Sree Narayana Colleges Sri. Vellappally Natesan, for providing us all the facilities for the completion of the work. We are indebted to the College Principal Prof. (Dr.) Nisha J. Tharayil for giving us the moral support for this endeavour. We extend our gratitude to the Head of the Department of Botany Dr. Nisha A P, who has been a constant source of support and encouragement. We thank the DBT Star College Scheme for the financial assistance provided for the successful completion of this work. Our colleagues in the Department of Botany supported us with their valuable suggestions. We thankfully acknowledge Ms. Smruthi S, Under Graduate student, Department of Botany (2020-23) for her meticulous drawings in the manual. Our family members always stood by us for the successful completion of the work. Last, but not the least we thank the Lord Almighty in guiding us throughout the work.

Authors

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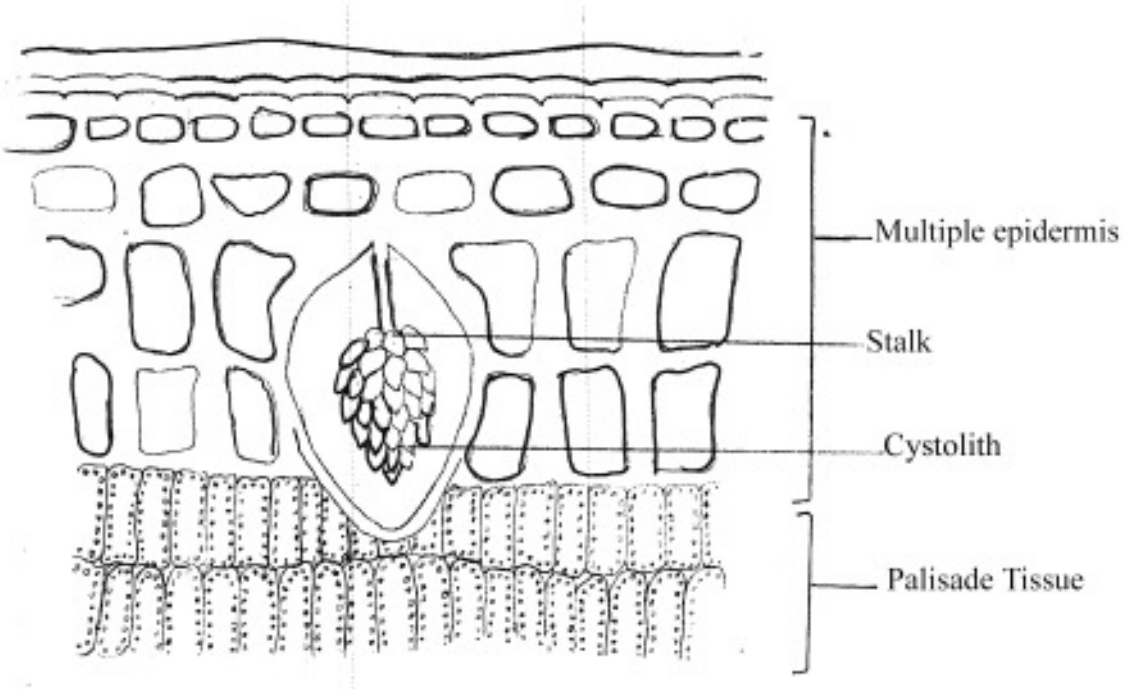
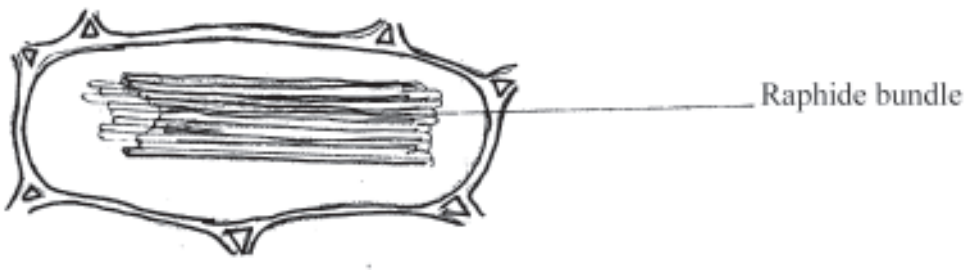
Mineral crystals

CYSTOLITH



RAPHIDES



MINERAL CRYSTALS

Mineral crystals are non-nitrogenous waste products. They occur either in the cell cavity or in the cell wall as calcium carbonate, calcium oxalate or silica

1. CYSTOLITH

1. Calcium carbonate crystals are commonly called Cystoliths.
2. Cystoliths develops as ingrowths from the wall of cells.
3. It forms as bunches of grapes.
4. They form a stalk - like protuberance from the cell wall and crystals of calcium carbonate are deposited over it.

Eg: *Ficus*

2. RAPHIDES.

1. They are thin, elongated and needle like crystals of calcium oxalate.
2. They are seen in specialized sac - like cells called raphide sacs.
3. They are also present in specialized cell known a crystal idioblasts.
4. They are also found as raphide bundles.

Eg : *Colocasia*

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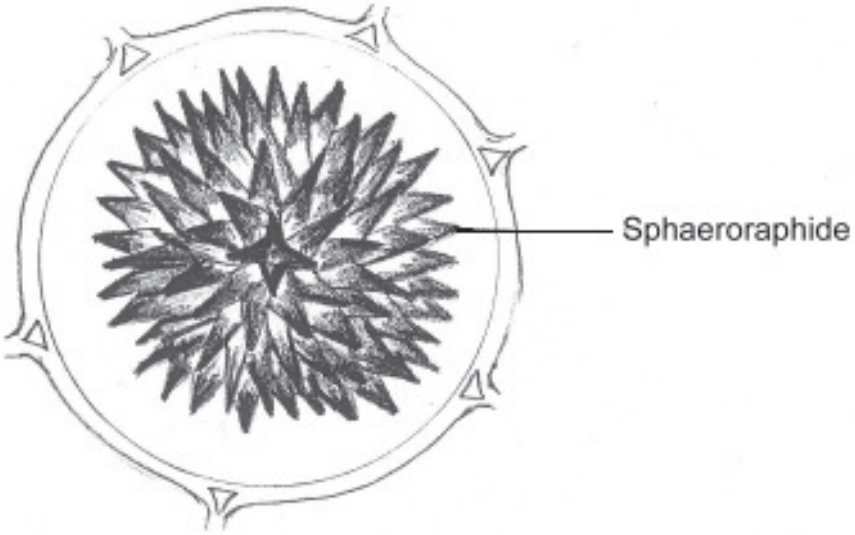
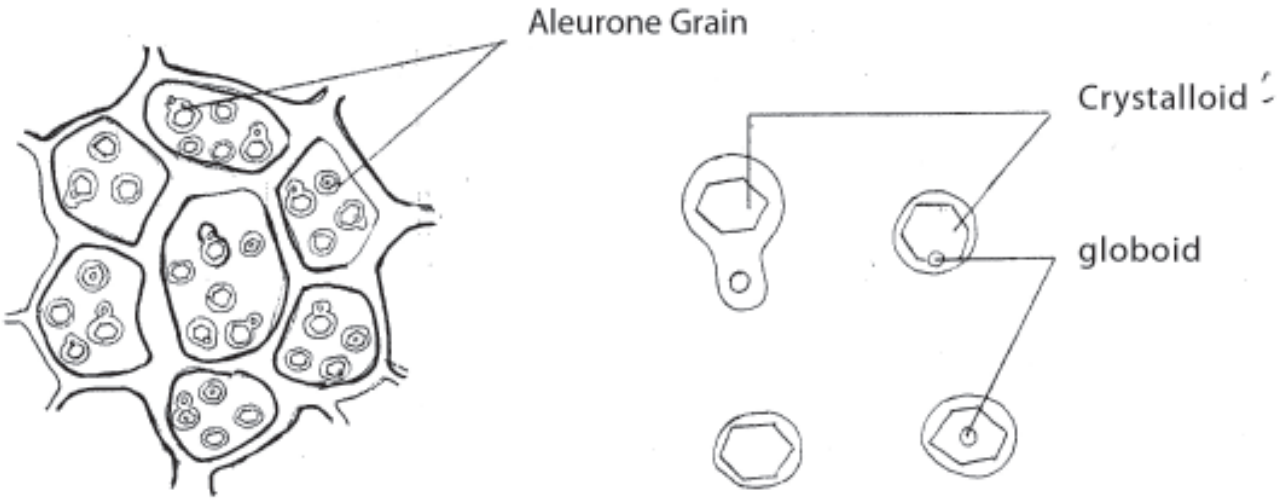
SPHAERORAPHIDES (DRUSES)



ALEURONE GRAINS



3. SPHAERORAPHIDES (DRUSES)

1. They are the globular, massive and needle - like compound crystals of calcium oxalate, formed around a central organic matter.
2. They have a rosette appearance.
3. They remain either attached to the cell wall or free in the cytoplasm in cortex, pith and phloem.

4. ALEURONE GRAINS

1. They are specialized dry vacuole for protein storage.
2. They are commonly seen in seeds, usually in the endosperm.
3. The cells containing aleurones form the aleurone layer.
4. Each grain consists of globoid and crystalloid bodies enclosed within a proteinaceous membrane.

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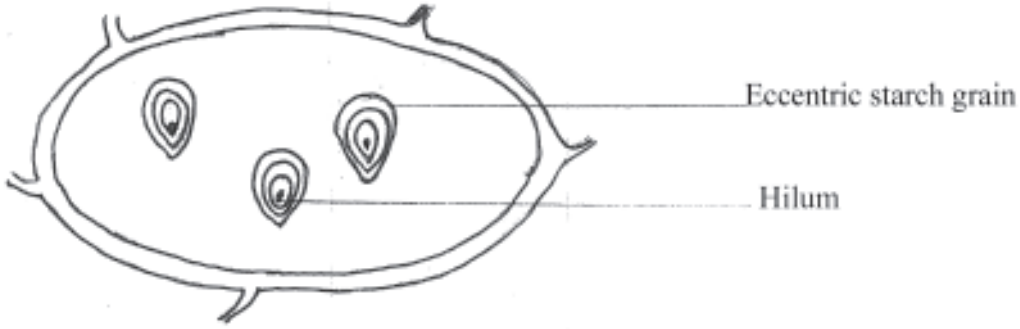
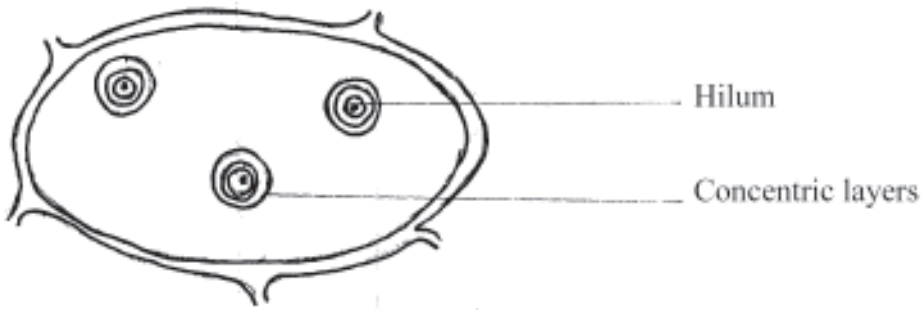
STARCH GRAINS

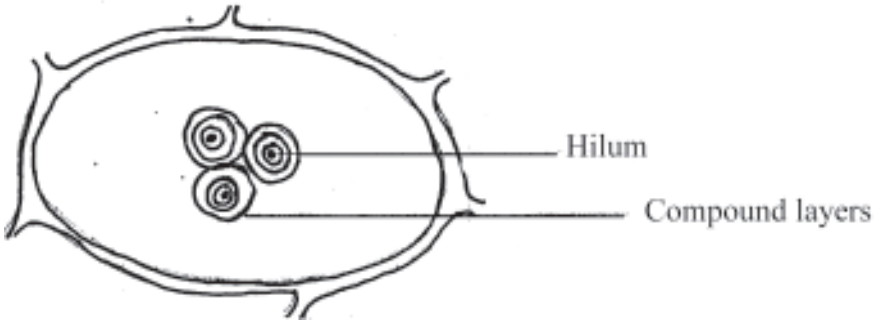
ECCENTRIC



CONCENTRIC



COMPOUND



STARCH GRAINS

Starch is a form of glucose containing reserve food material. Starch grains have layering around a central part called hilum. Based on the position of the hilum, they are of three types.

1. ECCENTRIC

1. Starch grain commonly shows distinct layer around a central point called hilum.
2. If the hilum is at one end it is called eccentric grain or if the starch layers are seen only in one side of the hilum it is called eccentric starch grain.
3. Found in potato tuber .

2. CONCENTRIC

1. Starch grain commonly shows a layer called hilum around a core point.
2. If the hilum is at the centre of the starch grain, it is called concentric starch grain.
3. Found in wheat grain.

3. COMPOUND

1. Two or more grains fuse together with more than one hilum.
2. Compound starch grains are found in *Oryza sativa.*

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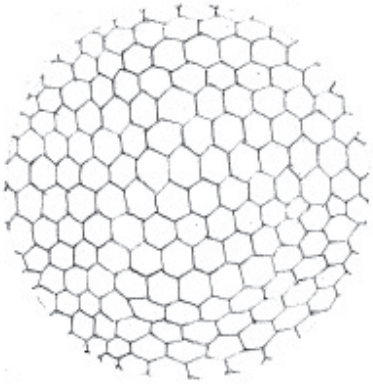
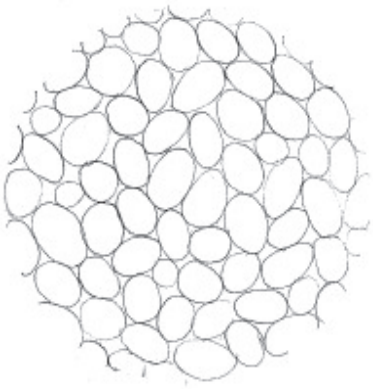
SIMPLE PERMANENT TISSUES

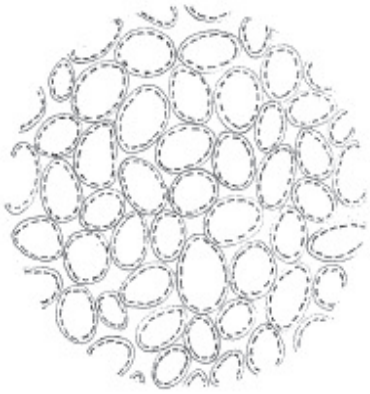
ANGULAR PARENCHYMA



ROUND PARENCHYMA

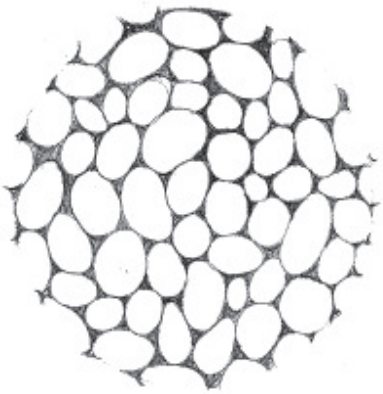


CHLORENCHYMA

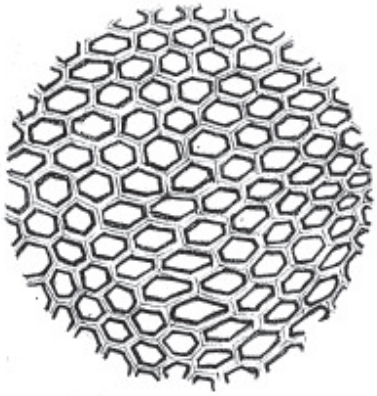


AERENCHYMA

COLLENCHYMA



SCLERENCHYMA



SIMPLE PERMANENT TISSUES

1. PARENCHYMA

1. It is the simplest and least specialized tissue.
2. It forms the fundamental or ground tissue of the plant body.
3. It consists of isodiametric, polygonal and thin - walled living cells, with or without intercellular spaces.
4. Parenchyma cells have the power to divide even at maturity.

2. AERENCHYMA

1. It is the air - storing parenchyma found in the roots, stems and leaves of hydrophytes.
2. The intercellular spaces are large and air-filled and are separated by partition walls in it.
3. It enables gas exchange and buoyancy to plants.

3. CHLORENCHYMA

1. It is the chlorophyll - containing photosynthetic parenchyma.
2. It is also called assimilatory parenchyma.
3. It is found in leaf mesophyll, sepals etc.

4. COLLENCHYMA.

1. It is the living supporting or mechanical tissue.
2. It is formed of elongated, thick - walled and terminally tapering cells.
3. Cell walls are non - lignified, irregularly thickened and formed of cellulose and pectin.
4. It shows localized thickening at the corners of the cells due to the extra deposition of cellulose, pectin and hemicellulose.

5. SCLERENCHYMA

1. It is the non - living mechanical tissue.
2. Cells are hard, thick - walled, elastic, dead and devoid of living protoplast at maturity.
3. The cells differ in size, shape and origin and are compactly arranged without intercellular spaces.
4. The cell walls are highly thickened, hard, lignified and pitted with cellulose and lignin deposits and low water content.

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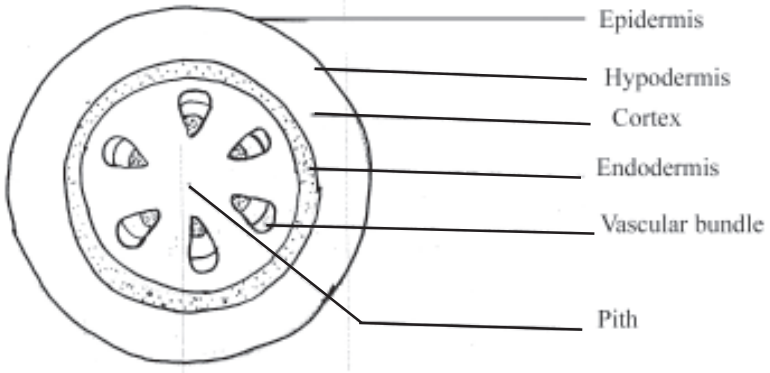
PRIMARY STRUCTURE OF DICOT STEM

GROUND PLAN



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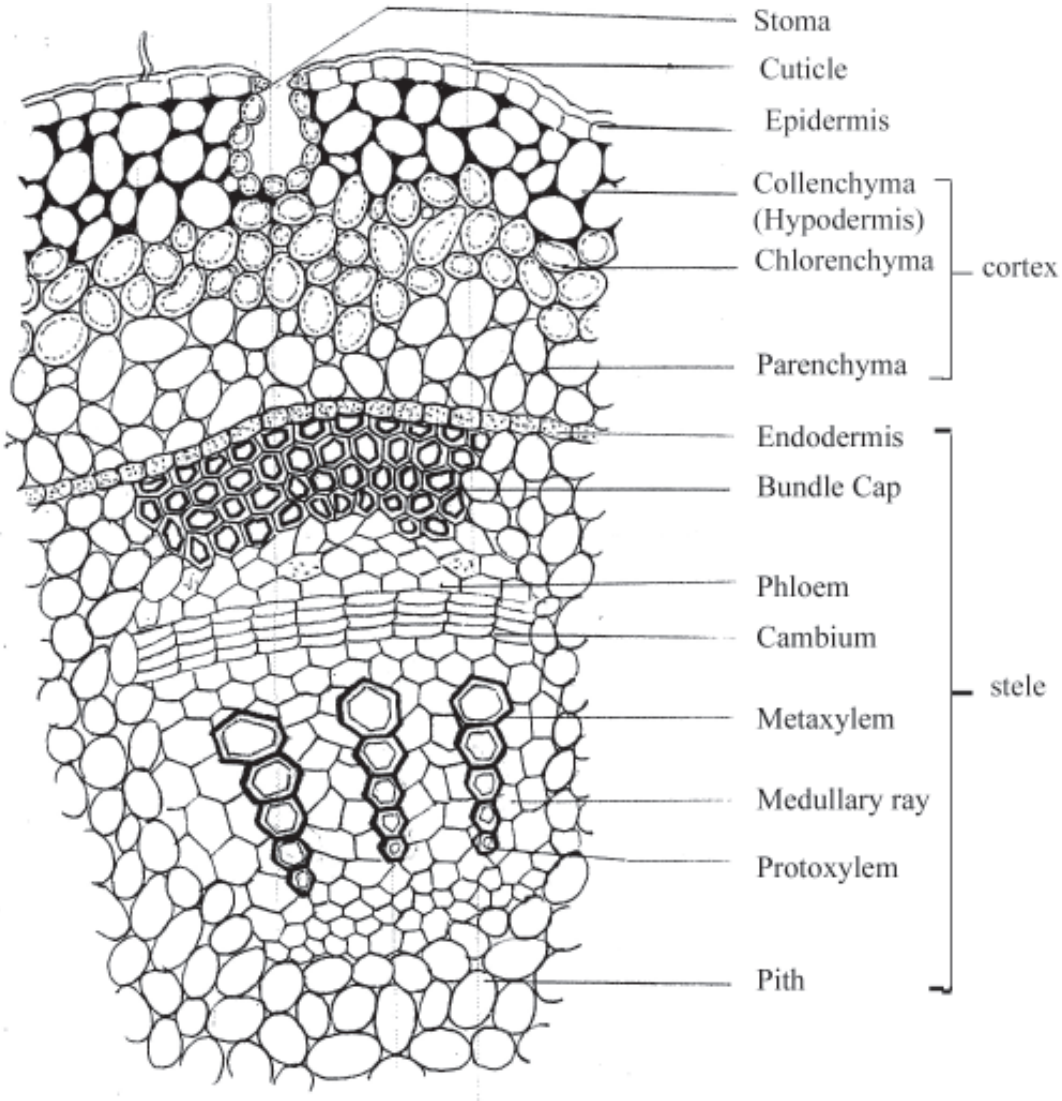

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PRIMARY STRUCTURE OF DICOT STEM

IDENTIFICATION FEATURES

1. Epidermis is covered with cuticle.
2. Multicellular epidermal hairs are present.
3. Heterogenous cortex with collenchyma, chlorenchyma and parenchyma cells.
4. Vascular bundles are collateral, conjoint and open.

5 Xylem is endarch.

Hence the given material is a stem

1 Hypodermis consists of 2-3 layers of collenchyma.

2 Vascular bundles are open, limited in number and arranged in the form of a broken ring.

3 Vascular bundles have sclerenchymatous bundle cap.

4 Xylem vessels are arranged linearly.

5 Pith and medullary rays are present.

6 Absence of secondary thickening .

Hence the given material is identified as primary structure of a dicot stem.

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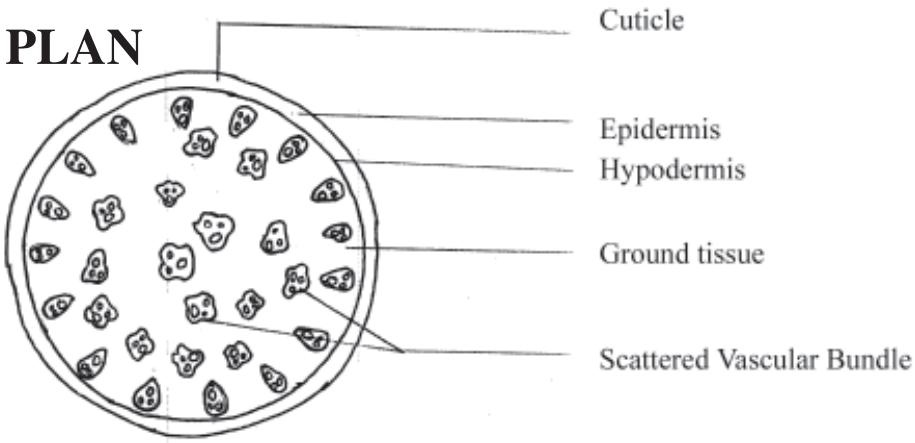
PRIMARY STRUCTURE OF MONOCOT STEM

GROUND PLAN



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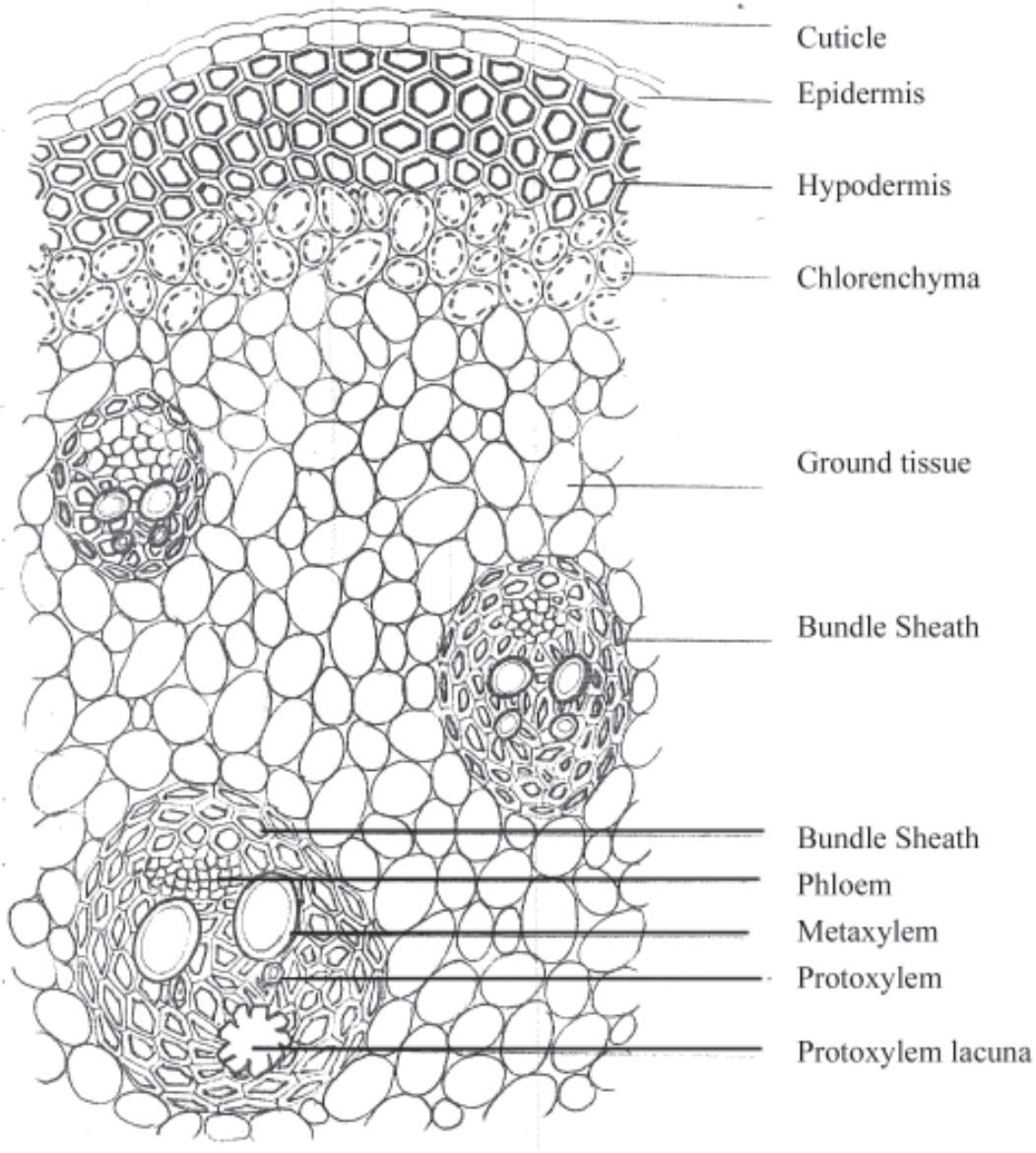

PRIMARY STRUCTURE OF MONOCOT STEM

IDENTIFICATION FEATURES

1. Epidermis is covered with cuticle.
2. Epidermal hairs are absent.
3. Ground tissue is not differentiated into cortex and stele.

4 Below the hypodermis, chlorenchyma is present.

5 Vascular bundles are collateral and conjoint.

6 Xylem is endarch.

Hence the given material is a stem

1 Hypodermis consists of 2-3 layers of sclerenchyma cells.

2 Vascular bundles are closed, numerous in number and scattered in the ground tissue.

3 Sclerenchymatous bundle sheath are present in vascular bundles.

4 Xylem vessels are few in number and arranged in 'V' or 'Y' shape.

5 Protoxylem lacuna is present.

6 Absence of secondary thickening.

Hence, the given material is identified as primary structure of a monocot stem.

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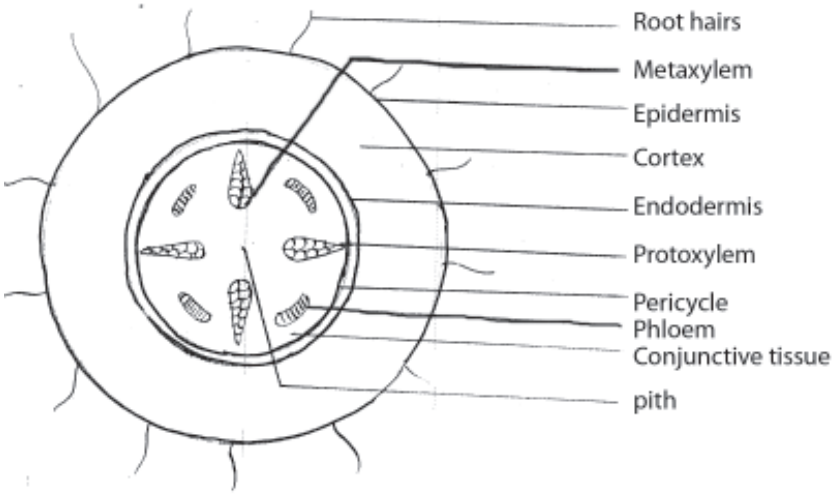
PRIMARY STRUCTURE OF DICOT ROOT

GROUND PLAN



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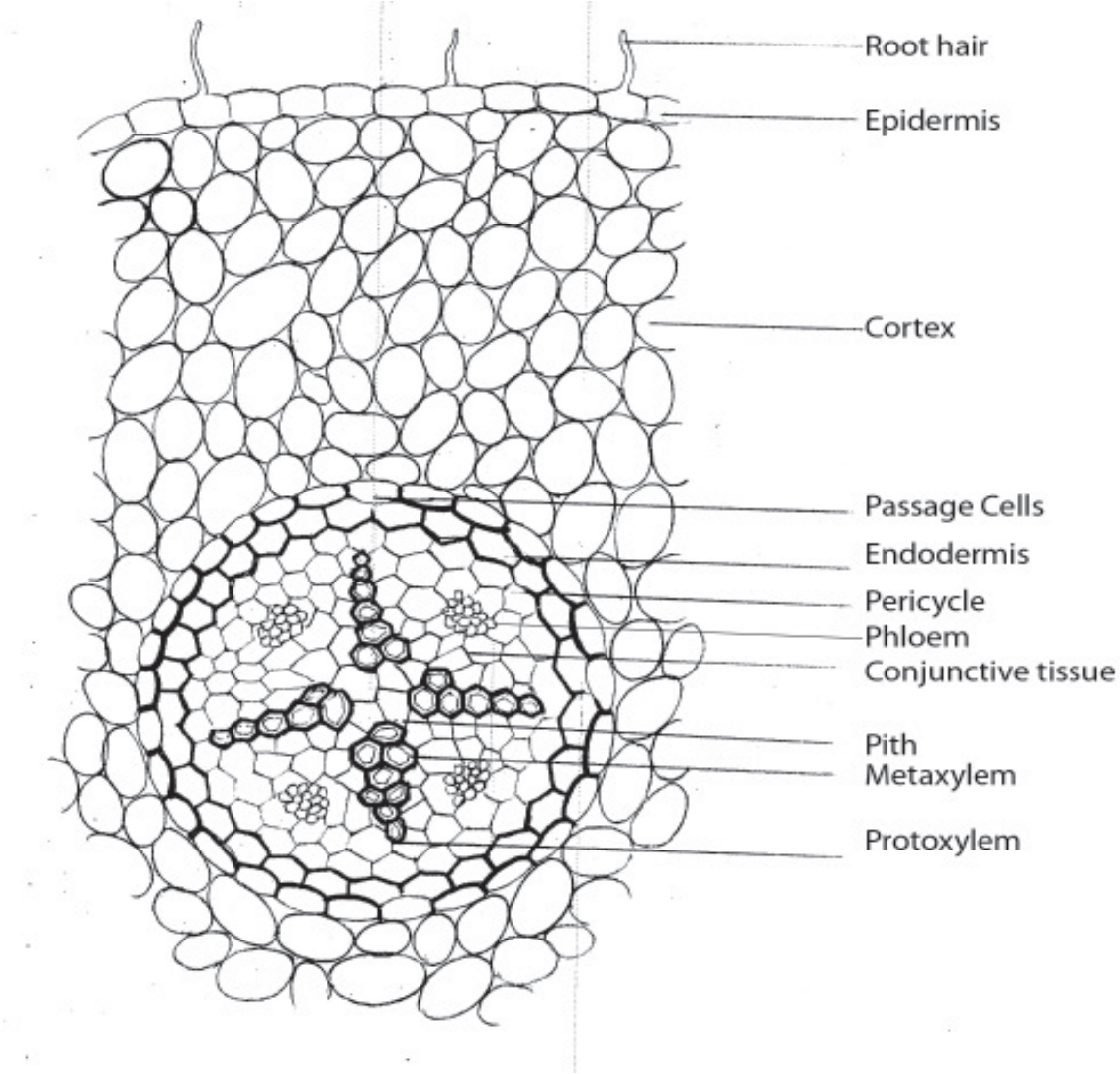

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PRIMARY STRUCTURE OF DICOT ROOT

IDENTIFICATION FEATURES

1. Epidermis is not covered with cuticle.
2. Unicellular epidermal hairs (root hairs) are present.
3. There is a homogeneous cortex composed of parenchymatous cells with intercellular spaces.
4. There is a distinct endodermis.
5. There is a single layered parenchymatous pericycle.
6. Passage cells are present in endodermis.
7. Xylem and phloem are arranged in a radial manner.
8. Xylem is exarch.

Hence the given material is a root

1. Vascular bundles are (4 in number or tetrarch condition).
2. Xylem vessels are polygonal in shape.
3. There is a reduced parenchymatous pith or pith is absent.

4 Absence of secondary thickening.

Hence the given material is identified as primary structure of a dicot root

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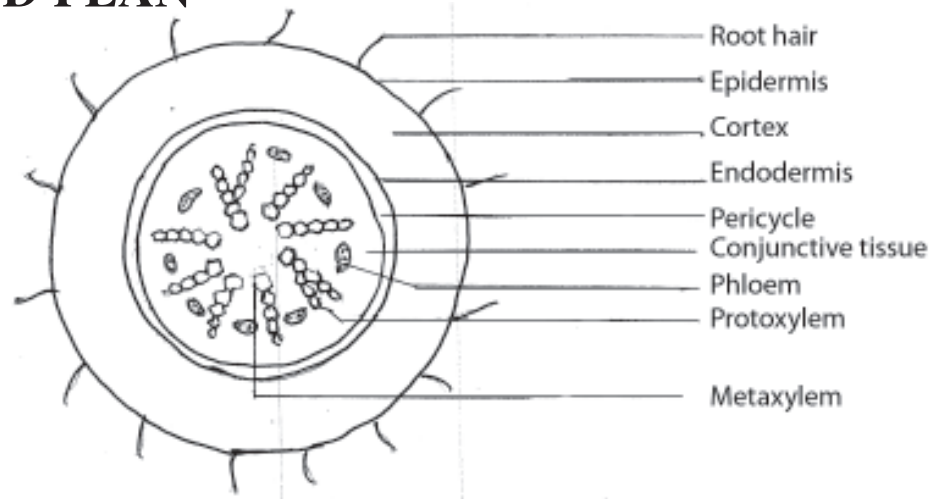
PRIMARY STRUCTURE OF MONOCOT ROOT

GROUND PLAN



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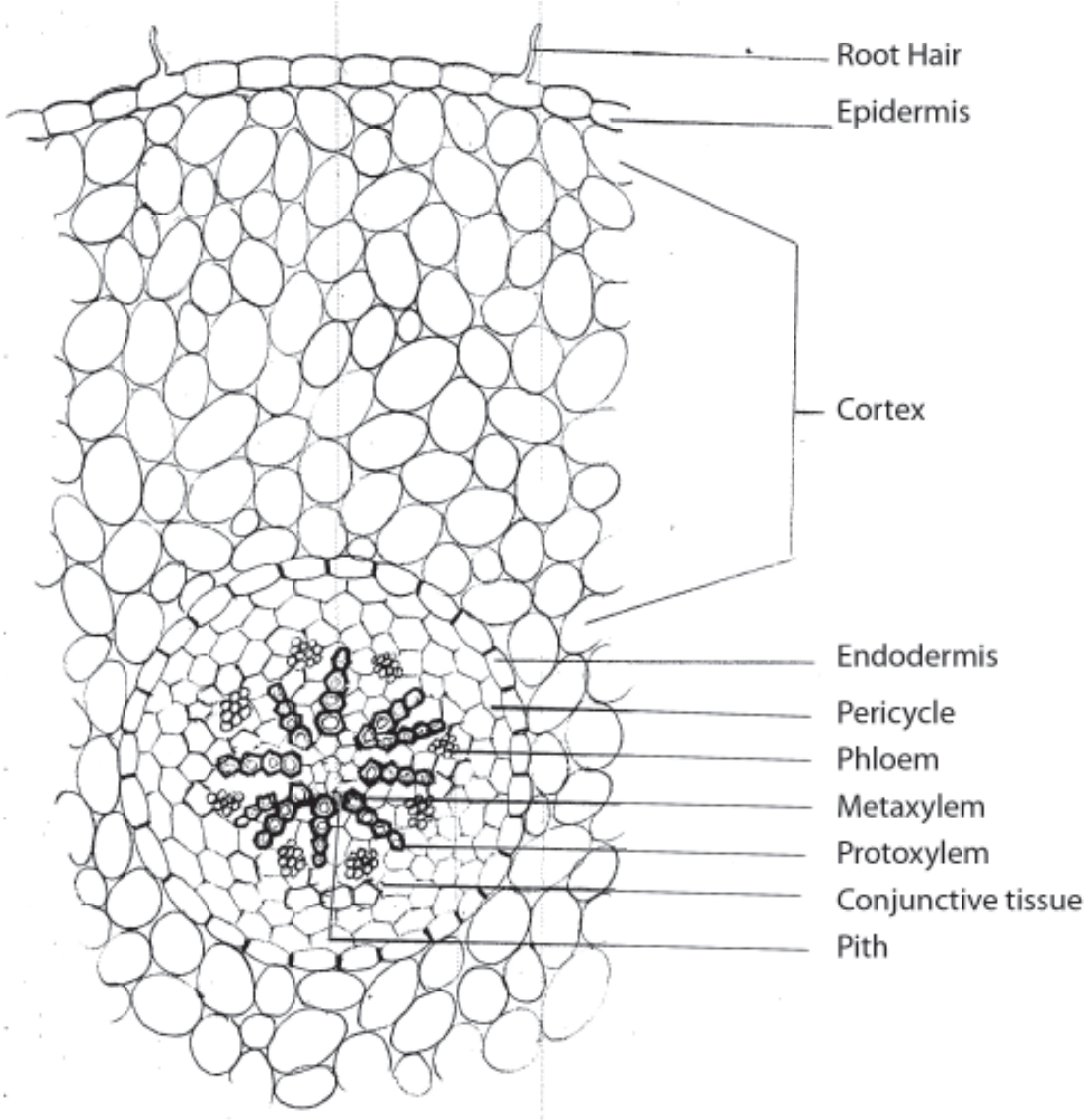

PRIMARY STRUCTURE OF MONOCOT ROOT

IDENTIFICATION FEATURES

1. Epidermis is not covered with cuticle.
2. Unicellular epidermal hairs (root hairs) are present.
3. There is a homogeneous cortex composed of parenchymatous cells with intercellular spaces and large air cavities.
4. There is a single layered endodermis and single layered parenchymatous pericycle.
5. Endodermis is clear with casparian thickening.
6. Passage cells are present in endodermis.
7. Xylem and phloem are arranged in radial manner.
8. Xylem is exarch in nature.

Hence the given material is a root

1. Numerous vascular bundles (7-10) are present.
2. Xylem vessels are round in shape.
3. Parenchymatous conjunctive tissue is present between xylem and phloem.
4. There is a large parenchymatous pith.

5 Absence of secondary thickening.

Hence the given material is identified as primary structure of monocot root.

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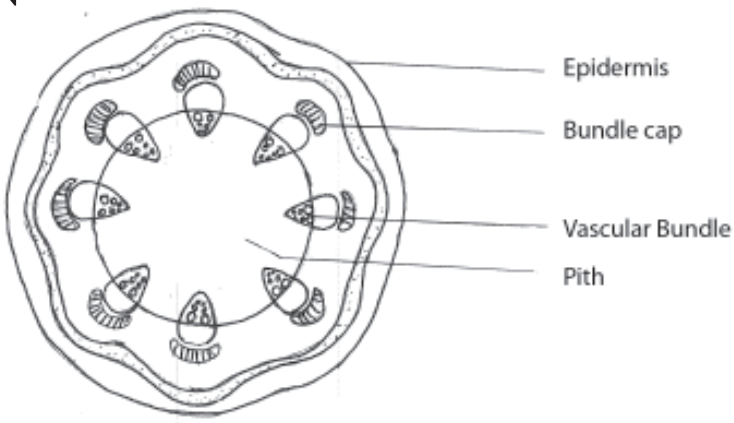
NORMAL SECONDARY STRUCTURE OF DICOT STEM - *VERNONIA*

GROUND PLAN



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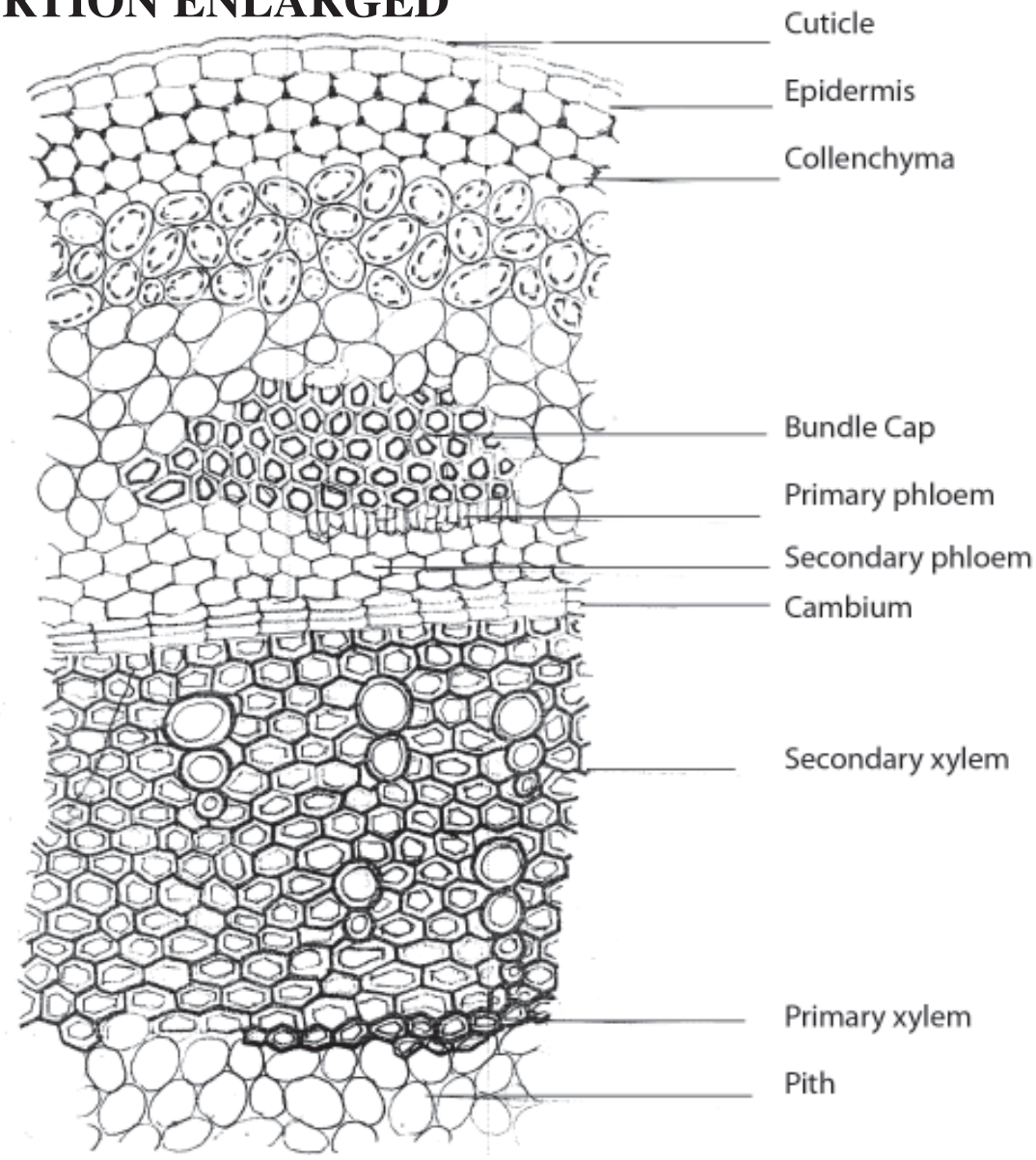

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NORMAL SECONDARY STRUCTURE OF DICOT STEM - *VERNONIA*

IDENTIFICATION FEATURES

1. Presence of endarch primary xylem.
2. Presence of epidermis covered with cuticle.
3. Presence of heterogeneous cortex with collenchyma and sclerenchyma.
4. Presence of conjoint and collateral vascular bundles.

Hence the given material is a stem.

1. Presence of conjoint, collateral and open vascular bundle.
2. Limited numbers of vascular bundles present.
3. Presence of pith.

Hence the given material is a dicot stem.

1. Presence of secondary xylem towards inside and secondary phloem towards outside due to the secondary thickening of cambial ring.
2. Primary xylem and secondary xylem seen in the large central parenchymatous pith as wedges.
3. Epidermis is replaced by periderm and lenticels due to the activity of cork cambium.

   Hence the given material is identified as normal secondary growth in a dicot stem.

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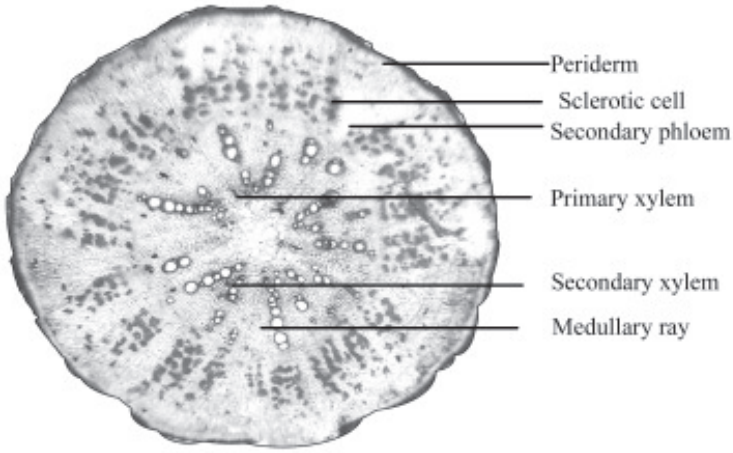
NORMAL SECONDARY STRUCTURE OF DICOT ROOT (*CARICA PAPAYA*)

GROUND PLAN



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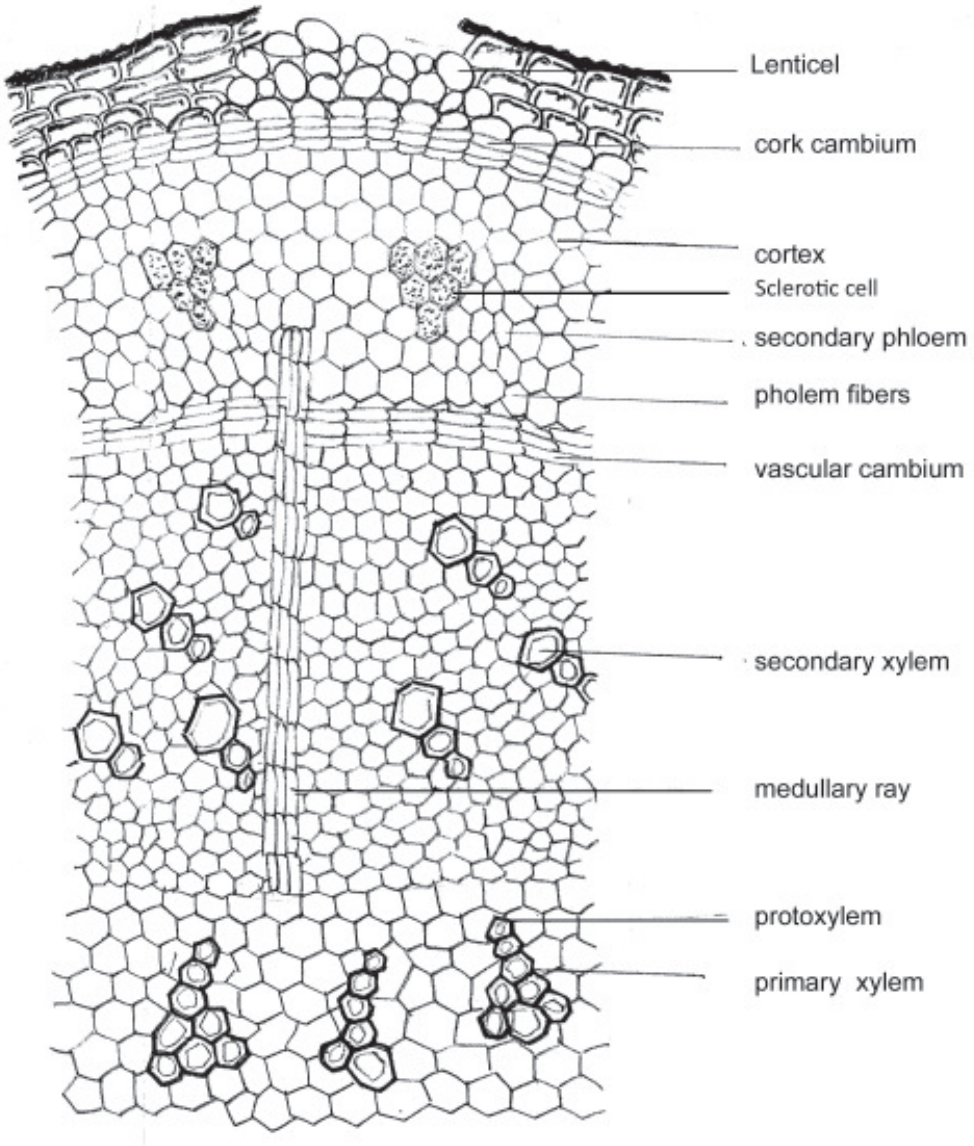


NORMAL SECONDARY STRUCTURE OF DICOT ROOT (*CARICA PAPAYA)*

IDENTIFICATION FEATURES

1. Presence of exarch primary xylem around the narrow pith
2. Presence of radial vascular bundles.
3. Presence of undifferentiated cortex.

Hence the given material is a root.

1. The xylem groups are limited in number.
2. Presence of small pith.
3. Presence of secondary growth.

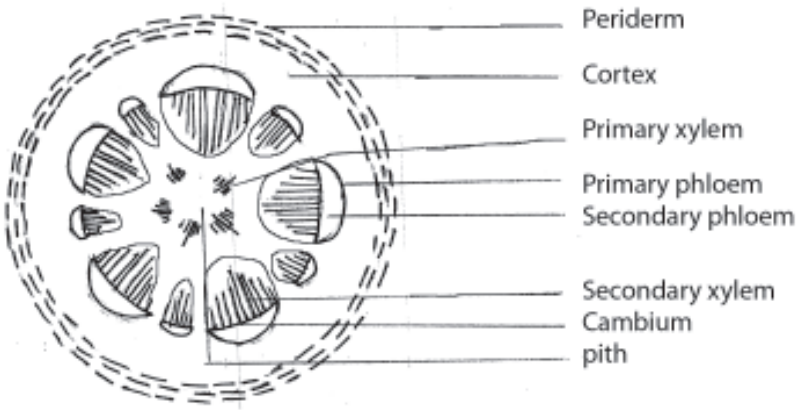
Hence the given material is a dicot root

1 Presence of secondary xylem and secondary phloem due to the secondary thickening of cambium.
2. Epidermis is replaced by periderm.
3. Presence of phellem, phellogen and phelloderm.
4. Presence of lenticels.

Hence the given material is identified as normal secondary growth in dicot root.

NORMAL SECONDARY STRUCTURE OF AERIAL DICOT ROOT (*TINOSPORA)*

GROUND PLAN



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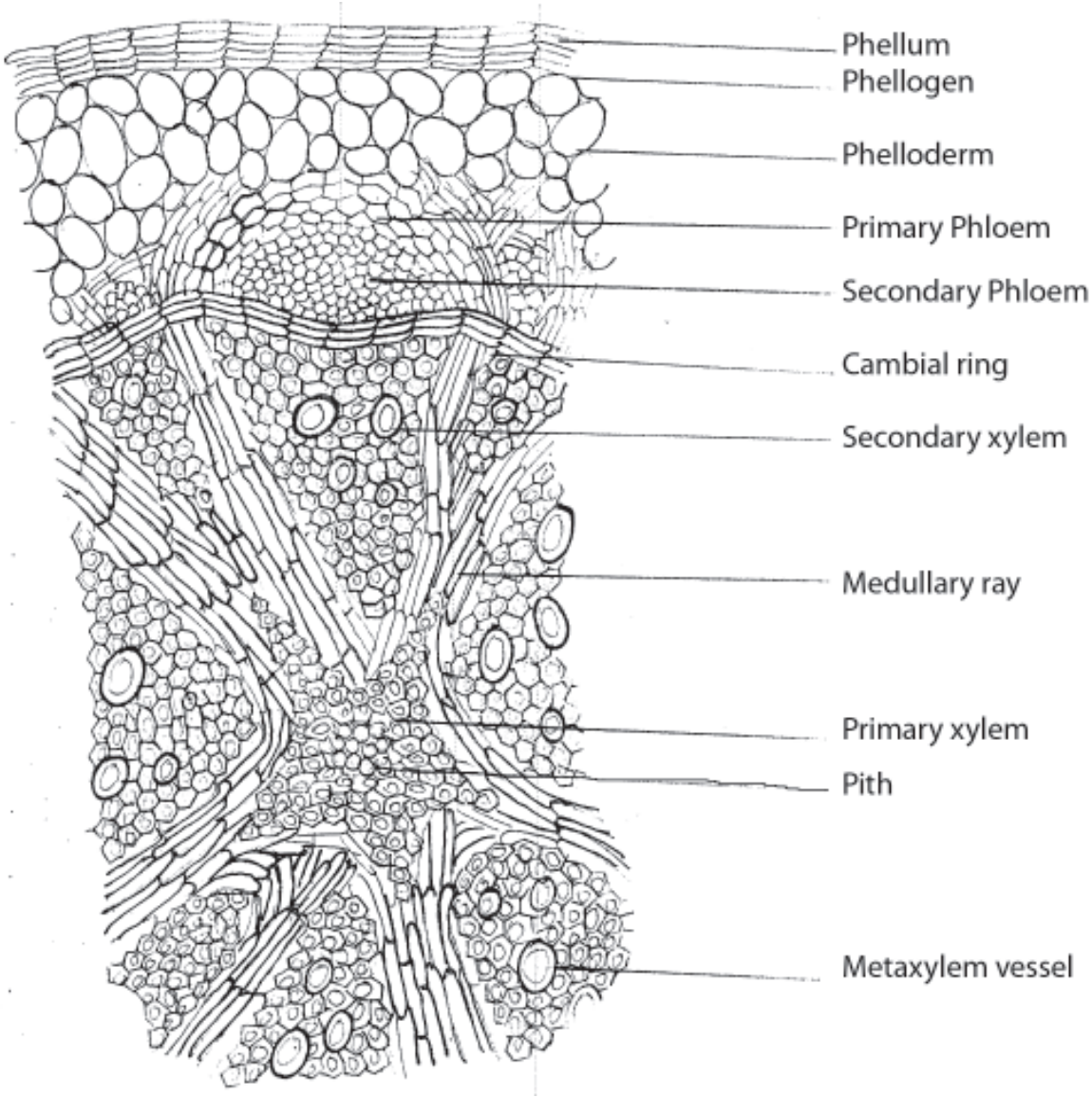

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NORMAL SECONDARY STRUCTURE OF AERIAL DICOT ROOT (*TINOSPORA)*

IDENTIFICATION FEATURES

1. Presence of exarch primary xylem around the narrow pith.
2. Presence of primary radial vascular bundles.

Hence the given material is a root.

1. Presence of limited number of primary xylem.
2. Presence of narrow pith.
3. Presence of cambium.
4. Presence of Secondary growth.

Hence the given material is a dicot root.

1 Presence of secondary xylem and secondary phloem produced by cambial activity.
2. Presence of periderm on the peripheral region.
3. Presence of well developed phellem, phellogen and phelloderm.
4. Presence of chloroplast containing cortical cells. Hence photosynthetic.
5. Epidermis ruptured due to secondary thickening.

Hence the given material is identified as normal secondary growth in aerial dicot root.

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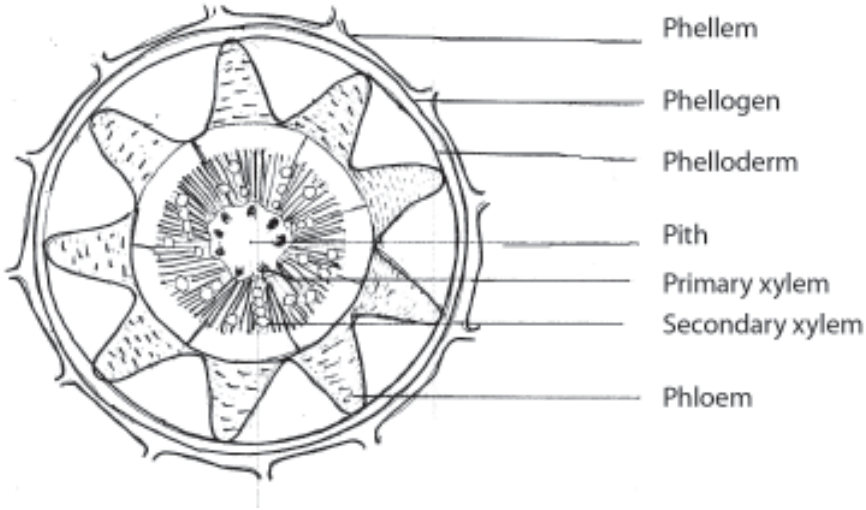
NORMAL SECONDARY STRUCTURE OF AERIAL DICOT ROOT- *FICUS*

GROUND PLAN



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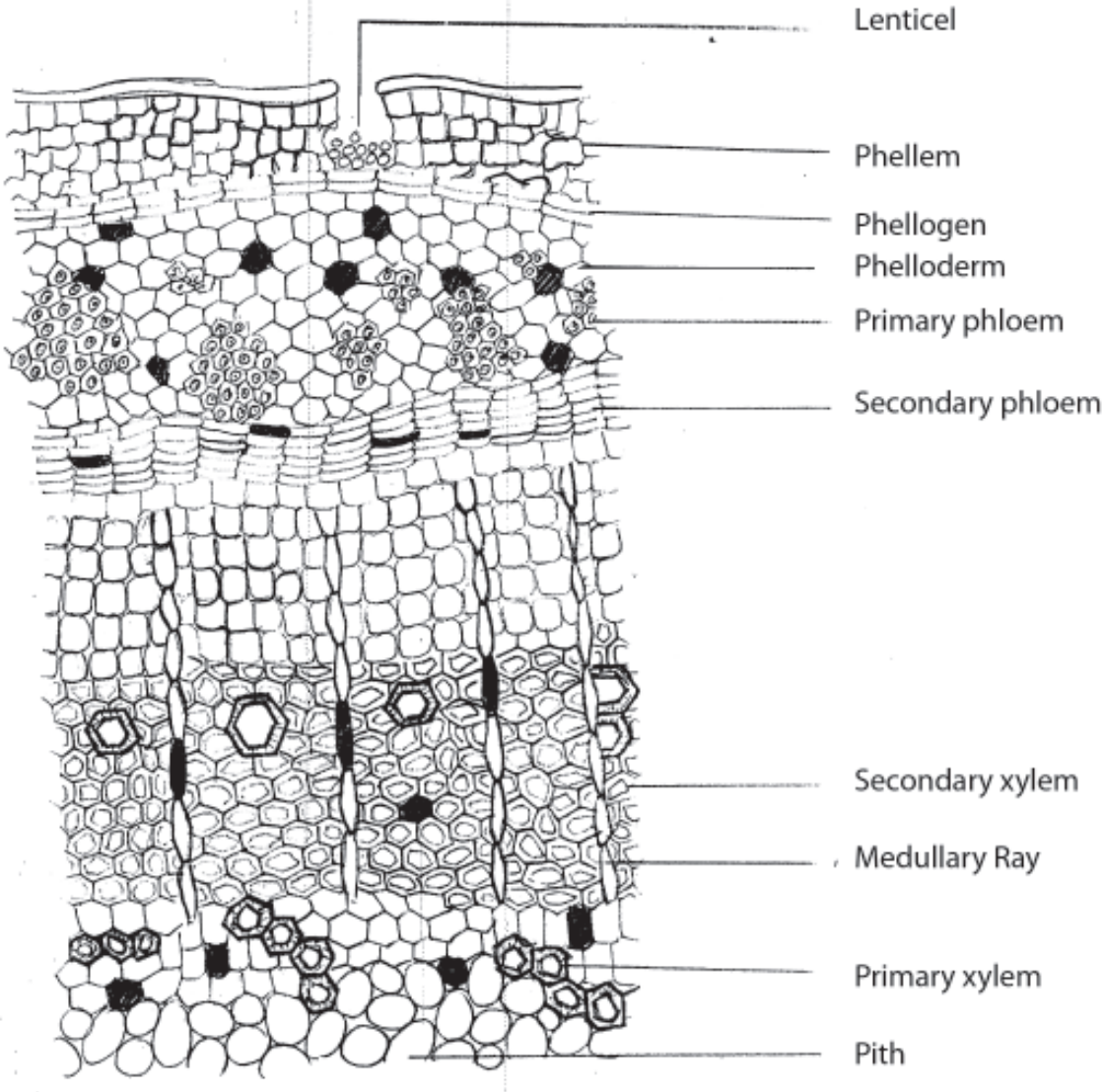


NORMAL SECONDARY STRUCTURE OF AERIAL DICOT ROOT- *FICUS*

IDENTIFICATION FEATURES

1. Presence of exarch primary xylem around the pith.
2. Presence of radial vascular bundles.
3. Presence of undifferentiated cortex .

Hence the given material is a root

1. Presence of limited number of vacular bundles..
2. Presence of reduced pith.

Hence the given material is a dicot root.

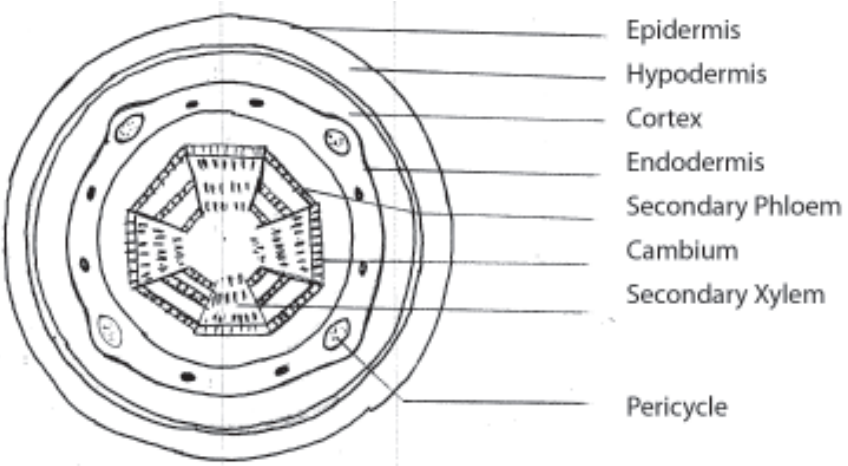
1. Presence of secondary xylem and secondary phloem due to cambial activity.
2. Epidermis is replaced by periderm.
3. Presence of phellem, phellogen and phelloderm
4. Presence of lenticels.

Hence the given material is identified as normal secondary growth in aerial dicot root.

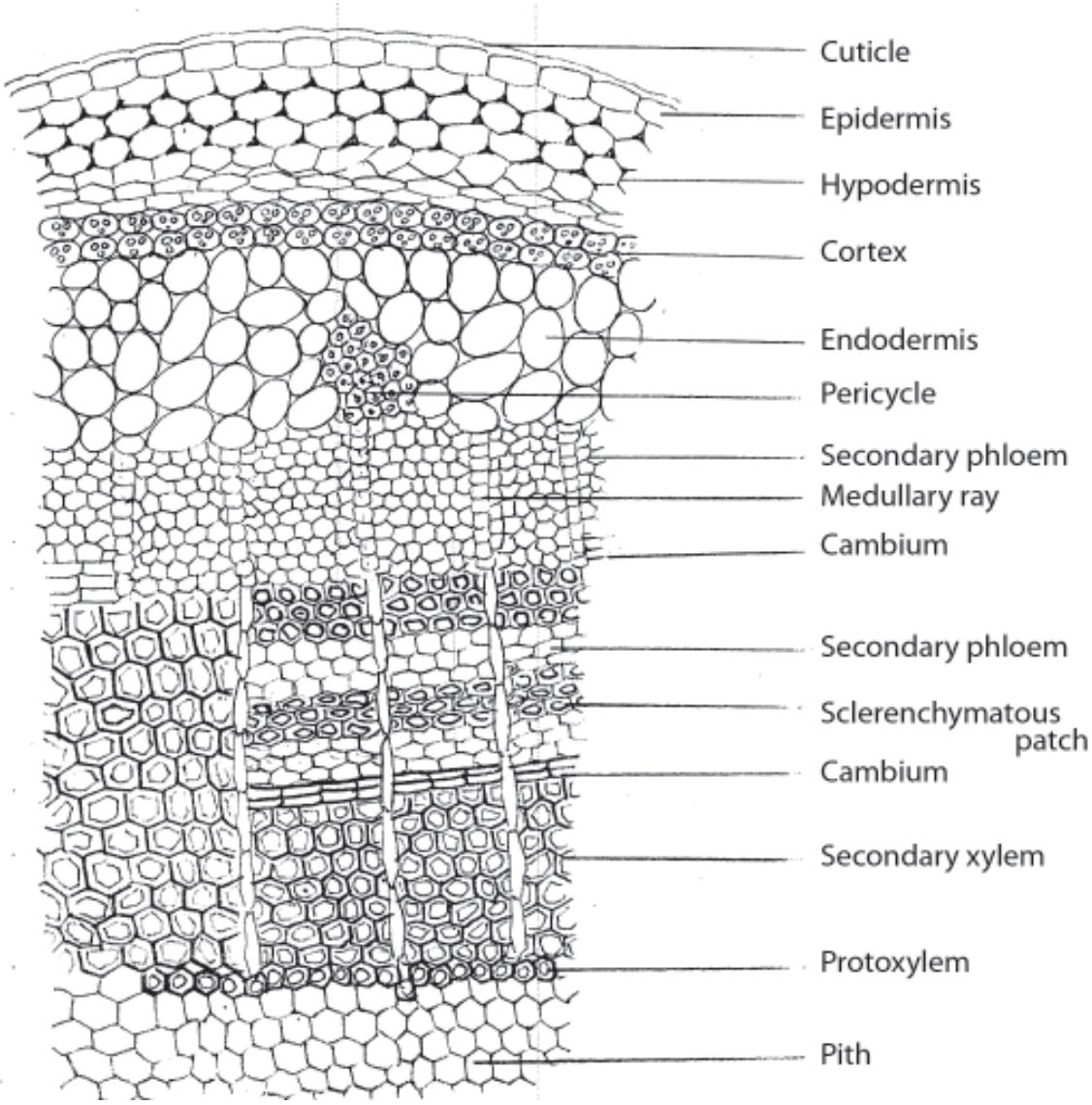
 ISBN 978-81-958369-0-1

ANOMALOUS SECONDARY STRUCTURE OF DICOT STEM - *BIGNONIA*

GROUND PLAN



A PORTION ENLARGED



ANOMALOUS SECONDARY STRUCTURE OF DICOT STEM - *BIGNONIA*

IDENTIFICATION FEATURES

1. Thick epidermis with cuticle.
2. Collenchymatous hypodermis is present.
3. Parenchymatous cortex is present.
4. Presence of conjoint, collateral vascular bundle.
5. Endodermis and pericycle is not well defined.
6. Presence of endarch primary xylem.

Hence the given material is a stem.

1. Presence of conjoint, collateral and open vascular bundles.
2. Vascular bundles are limited in number.
3. Vascular bundles are arranged in a broken ring manner.
4. Well defined pith is present.

Hence the given material is a dicot stem.

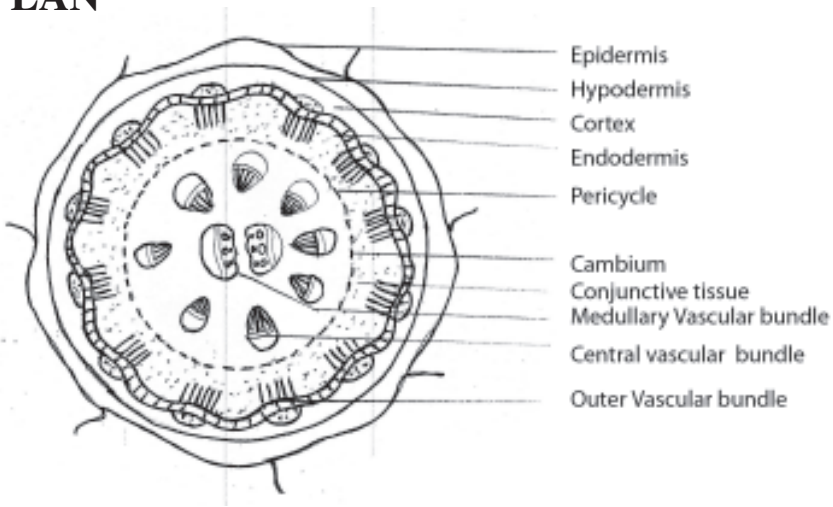
1. Abnormal functioning of the cambium is seen.
2. It possess 4 large sized and small sized wedges of secondary phloem perforating into secondary xylem.
3. The secondary phloem has sclerenchymatous patches.
4. The extrastelar growth is normal which results in the formation of periderm.

Hence the given material is identified as anomalous secondary thickening in dicot stem- *Bignonia.*

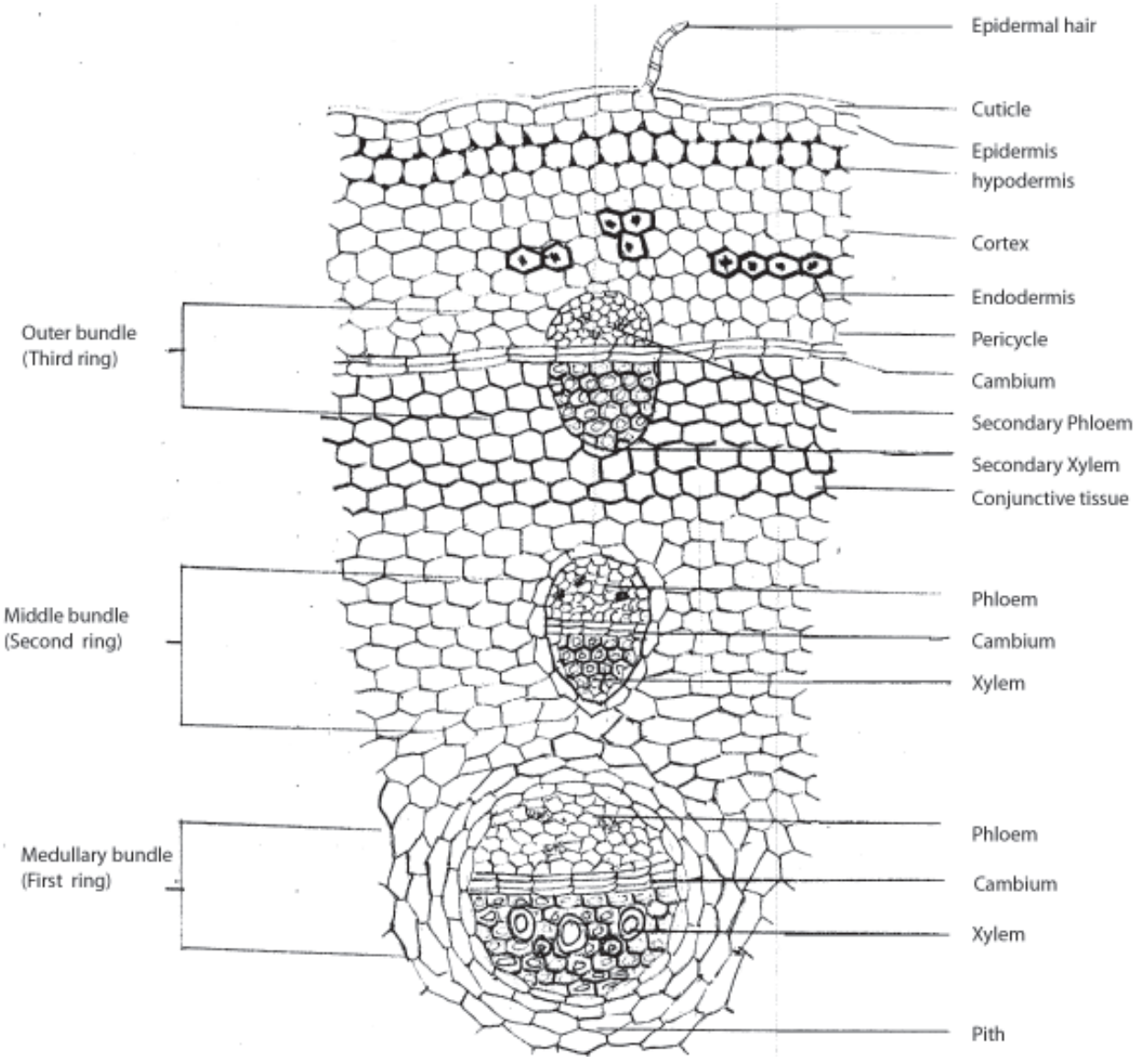
 ISBN 978-81-958369-0-1

ANOMALOUS SECONDARY STRUCTURE OF DICOT STEM- *BOERHAVIA*

GROUND PLAN



A PORTION ENLARGED



ANOMALOUS SECONDARY STRUCTURE OF DICOT STEM- *BOERHAVIA*

IDENTIFICATION FEATURES

1. Presence of single layered epidermis with cuticle.
2. Presence of collenchymatous hypodermis.
3. Presence of parenchymatous cortex.
4. Presence of endodermis and pericycle.
5. Xylem is endarch.
6. Vascular bundles are conjoint and collateral.

Hence the given material is a stem

1 Presence of 3 rings of vascular bundles.

2 Vascular bundles are conjoint, collateral and open.

Hence the given material is a dicot stem

1. Presence of more than one ring of cambia.
2. Abnormal functioning of cambia with formation of conjunctive tissue is seen.

3 Presence of unusual positioned extra stelar cambial ring.

4 Presence of central two medullary bundles, middle bundles and outer smaller bundles.

5 Presence of cork and periderm.

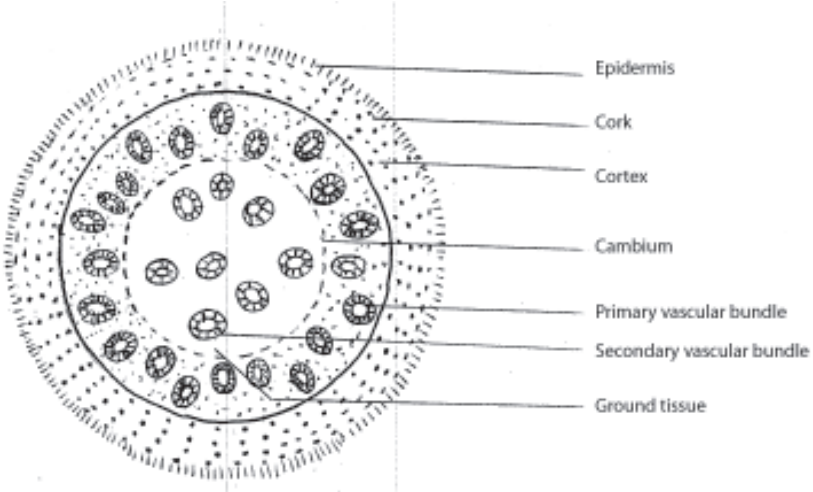
6 Pith is reduced.

Hence the given material is identified as anomalous secondary thickening in dicot stem- *Boerhavia*

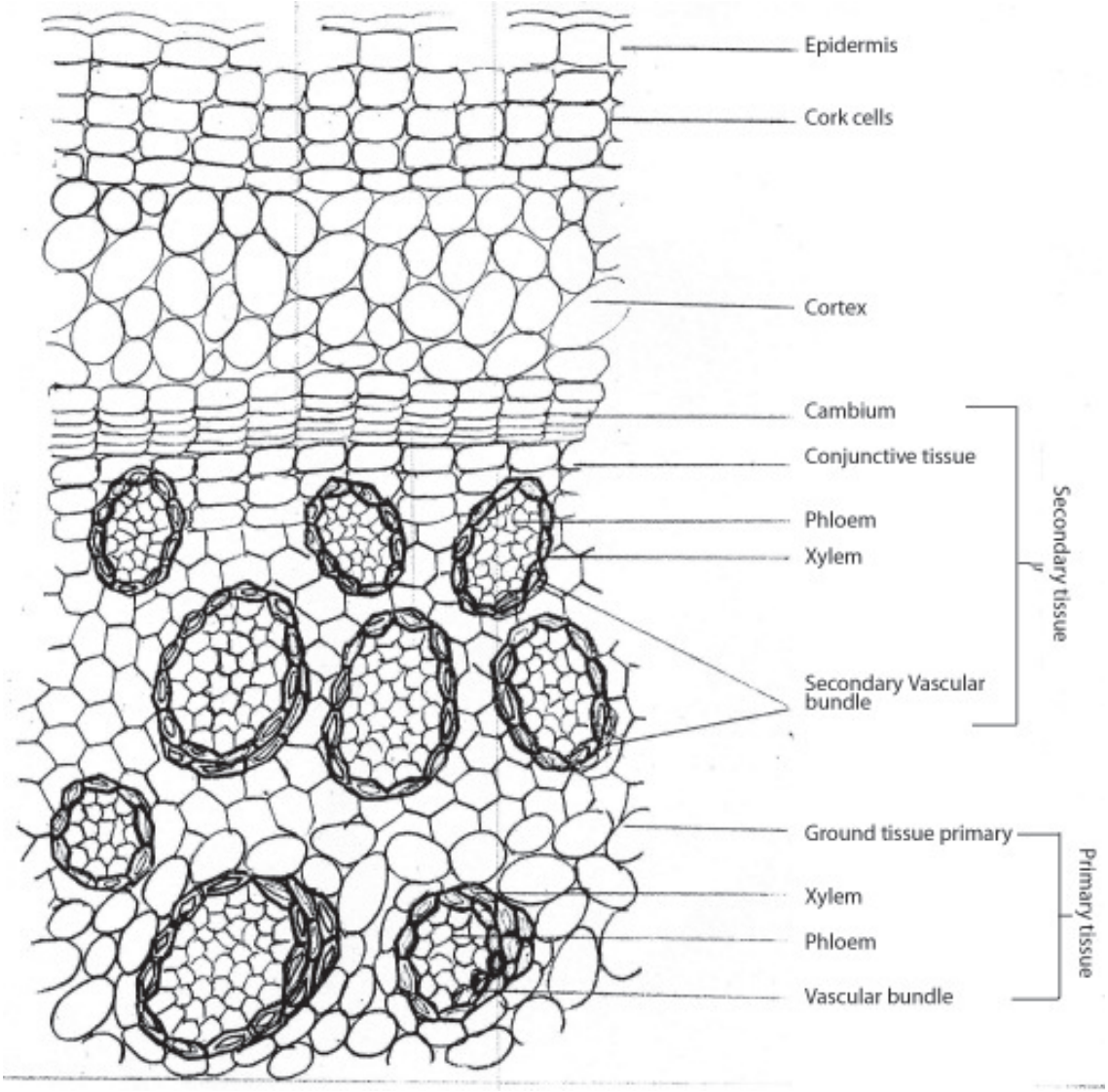
 ISBN 978-81-958369-0-1

ANOMALOUS SECONDARY STRUCTURE OF MONOCOT STEM - *DRACAENA*

GROUND PLAN



A PORTION ENLARGED



 ISBN 978-81-958369-0-1

ANOMALOUS SECONDARY STRUCTURE OF MONOCOT STEM - *DRACAENA*

IDENTIFICATION FEATURES

1. Presence of outermost epidermis with thick cuticle.
2. Vascular bundles are conjoint, collateral.
3. Xylem is endarch.

Hence the given material is a stem

1 Vascular bundles are closed and scattered in the ground tissue.

2. Sclerenchymatous hypodermis is present.

3 Vascular bundles are closed, collateral and conjoint embedded in the parenchymatous ground tissue.

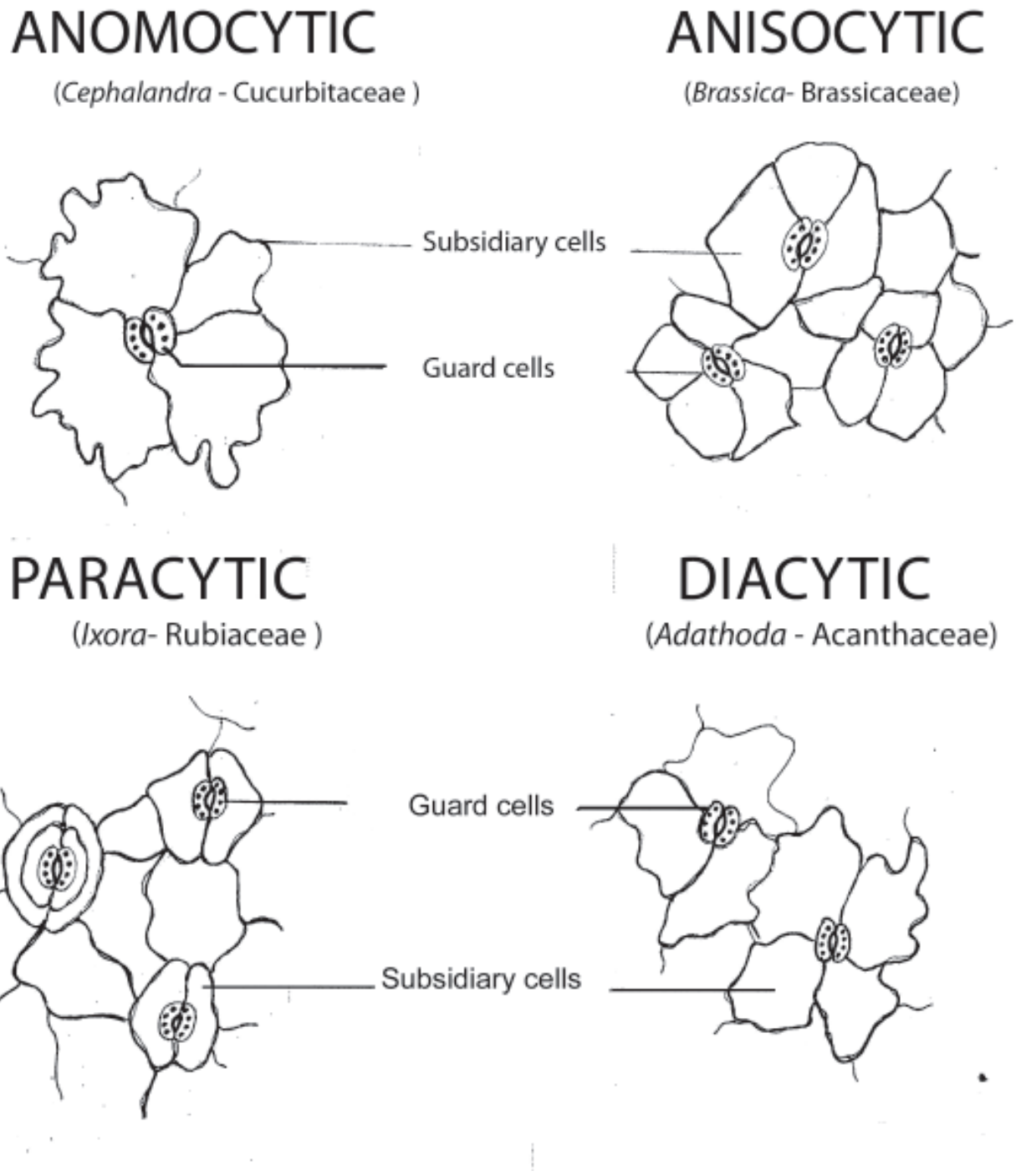
Hence the given maerial is a monocot stem.

1. Abnormal functioning of cambium is seen.
2. Presence of cortical cells external to vascular bundle.
3. Presence of amphivasal vascular bundle.
4. Cambium is present.
5. Presence of alternate rows of vascular bundles.
6. Cork cells formed from cork cambium is seen on the outside.

Hence the given material is identified as anomalous secondary thickening in monocot stem- *Dracaena.*

 ISBN 978-81-958369-0-1

TYPES OF STOMATA



 ISBN 978-81-958369-0-1

TYPES OF STOMATA

Stomata are minute openings in epidermis delimited by two guard cells. Guard cells with the opening forms the stoma. The stoma with the adjacent subsidiary cells forms the stomatal apparatus. The different types are :

1. **Anomocytic (irregular - celled) Stomata**

1. The stoma is surrounded by a definite number of subsidiary cells that are indistinguishable from other epidermal cells.
2. They are also known as irregular- celled type or Ranunculaceous type.

2. **Anisocytic (unequal - celled) Stomata.**

1. There are three subsidiary cells of which one is small and the other two are relatively larger.
2. They are also known as the unequal celled or Cruciferous type.

3. **Paracytic (parallel - celled) Stomata**

1. The stoma is accompanied on either side by one or more subsidiary cells which lie parallel to the long axis of the stomatal pore and guard cells.
2. They are also known as the parallel celled or Rubiaceous type.

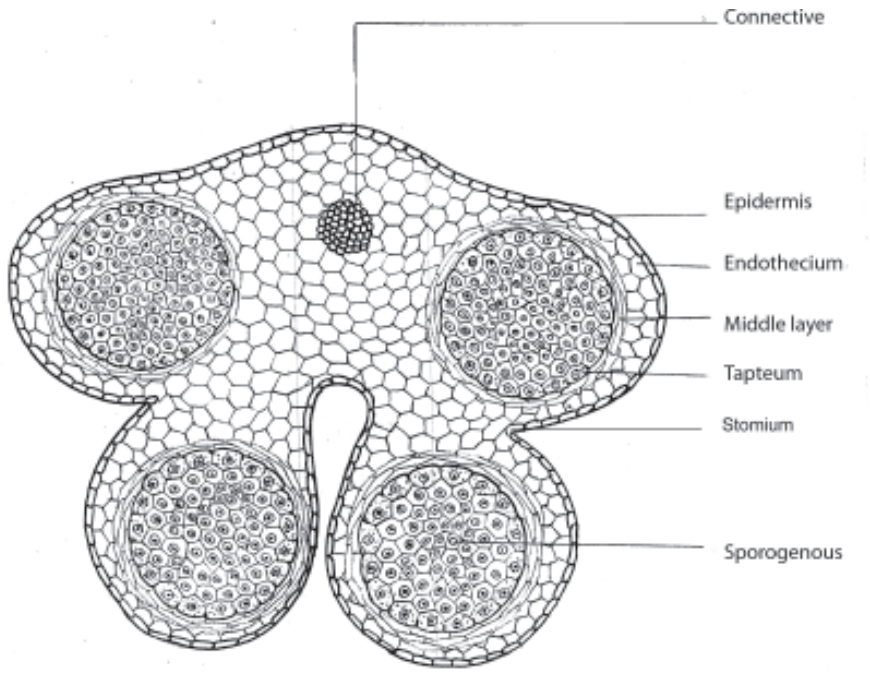
4. **Diacyctic (cross - celled) stomata**

1. Each stoma is enclosed by a pair of subsidiary cells whose common wall is at right angles to the long axis of the guard cells.
2. They are also known as cross celled type or Caryophyllaceous type.

REPRODUCTIVE BOTANY

 ISBN 978-81-958369-0-1

YOUNG ANTHER



 ISBN 978-81-958369-0-1

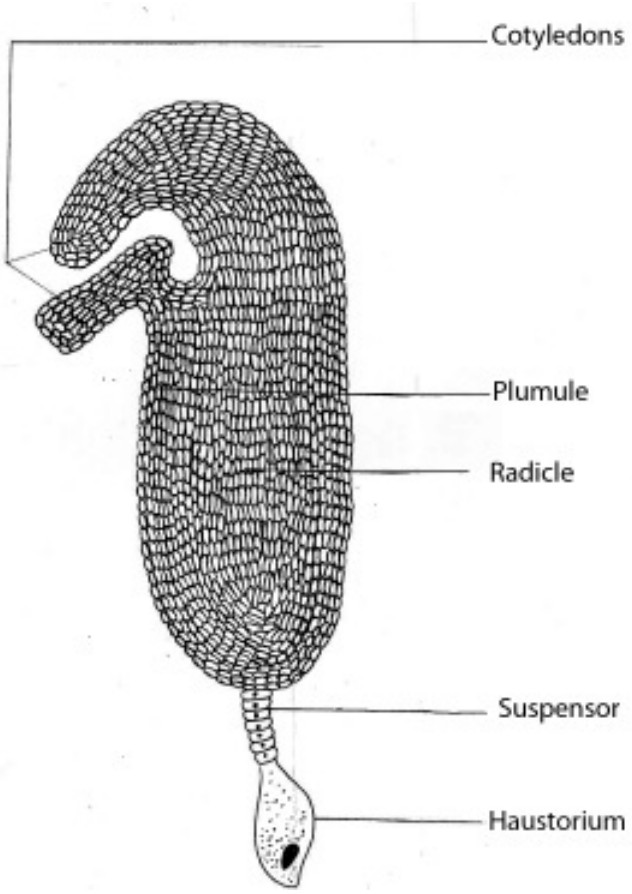
ANTHER

Anther is the lobed part of microsporophyll bearing pollen grains or microspores. It contains sac like structures called microsporangium or pollen sac that bears microspore mother cells. Microspore mother cells undergo meiosis to give rise to four haploid microspores each. They undergo further division to form mature pollen grains.

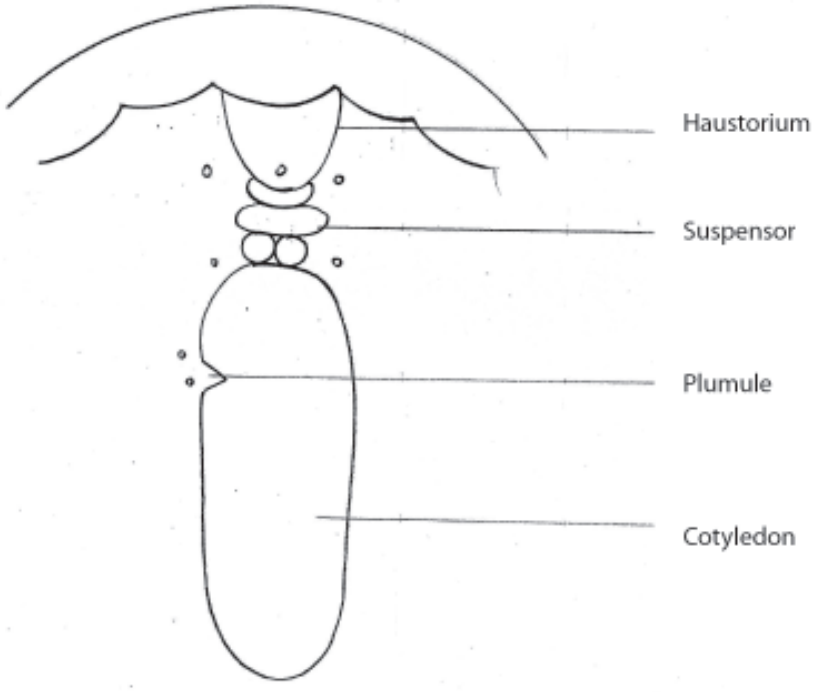
Internal Structure of a mature Anther

A mature anther has four layered wall. Outermost epidermis, sub- epidermal fibrous endothecium, 3-4 tiers of middle layer and innermost tapetum. Tapetum provides nourishment to the developing pollen grains present inside the microsporangia. The anther is connected to the filament through connective. On maturation of the anther, only epidermis and endothecium persists. It undergoes dehiscence through transverse slit, longitudinal slit, pores etc. to release the pollen grains with the help of stomium.

DICOT EMBRYO



MONOCOT EMBRYO



EMBRYO

Embryo is the diploid multicellular structure formed from zygote that represents sporophytic generation of Angiosperms. It develops to form sporophyte. Common features of an embryo are the presence of haustorium, suspensor, cotyledon, plumule and radicle. Based on the number of cotyledons present, they are classified into two *viz*., dicot embryo and monocot embryo.

1. Dicot Embryo

Dicot embryo has two lateral cotyledons flanking the terminal plumule. The embryo takes nourishment from the endosperm through haustorium. Suspensor helps to push the haustorium into the endosperm for effective absorption. Radical forms root system of the mature sporophyte and plumule develops to shoot system. Coleoptile and Coleorrhiza are absent. The different stages of dicot embryogenesis are globular stage, cordate stage and torpedo stage.

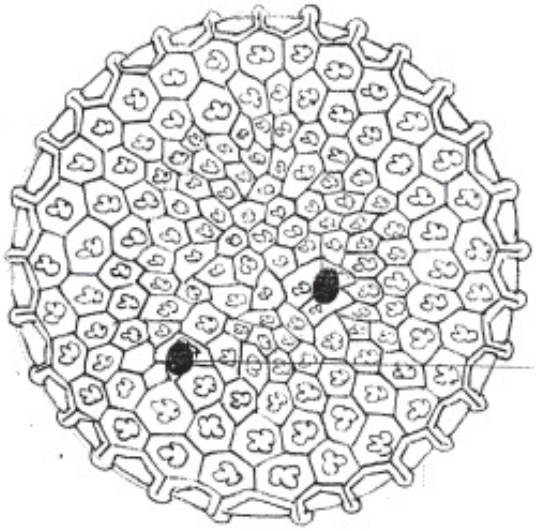
2. Monocot Embryo

Monocot embryo has a single terminal cotyledon called Scutellum. Plumule is lateral and surrounded by coleoptile. Radicle is lateral and protected by a root cap and Coleorrhiza. Suspensor helps to push the haustorium into the endosperm for effective absorption. Radical forms root system of the mature sporophyte and plumule develops to shoot system. The different stages of monocot embryogenesis are globular stage, scutellar stage and coleoptile stage.

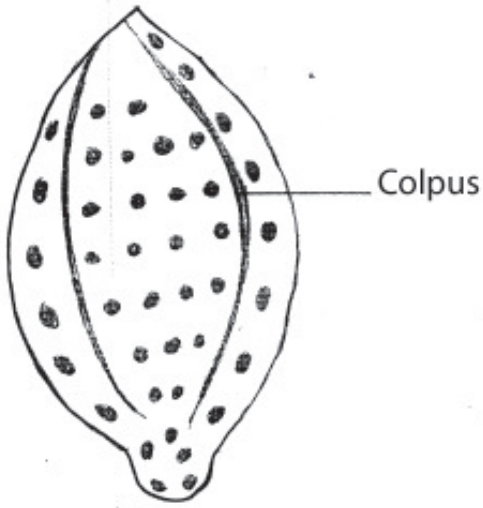
PALYNOLOGY

 ISBN 978-81-958369-0-1

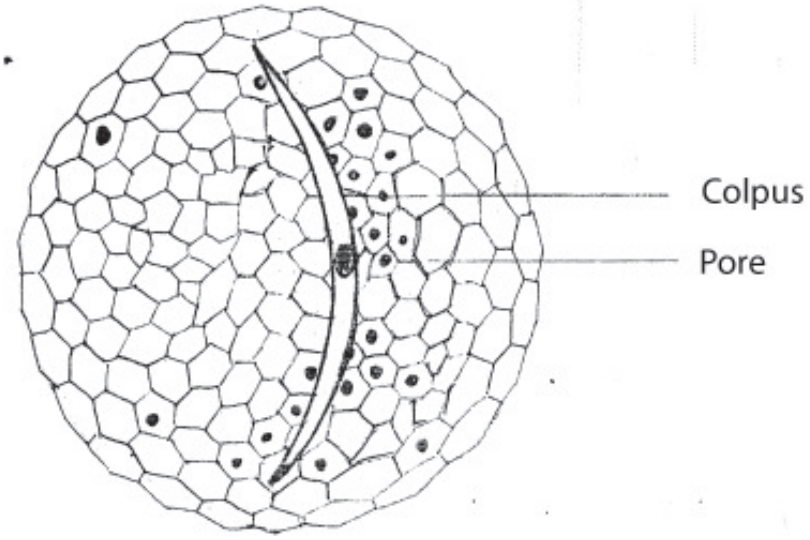
PORATE POLLEN



COLPATE POLLEN



COLPORATE POLLEN



POLLEN GRAINS

Pollen grains or microspores represent the male gametophytic generation of Angiosperms. They are formed from pollen mother cells through meiotic division. They are protected by a strong wall called Sporoderm. The pollen wall has two layers namely outer exine and inner intine. Based on the shape of the aperture on exine, there are three types of pollen grains:

1. **Porate pollen** : The apertures on porate pollen are pores. The number of pores on a pollen varies with species. They represent the area of germination of the pollen grain after pollination. The area bordered by two pori is the mesoporium.

2. **Colpate pollen** : The apertures on Colpate pollen are elongated furrows. The number of furrows on a pollen varies with species. They represent the area of germination of the pollen grain after pollination. The area bordered by two colpi is the mesocolpium.

3. **Colporate pollen**: The apertures on such pollen grains has elongated furrow with a circular pore in the centre. The number of apertures on a pollen varies with species. They represent the area of germination of the pollen grain after pollination.

DATA HANDLING IN SCIENCE

FREQUENCY DISTRIBUTION

Characteristics of Frequency distribution

1. The data are simultaneously collected at a point of time so that the time element is not a variable.
2. The classification is made according to the magnitude of the variable rather than its quantitative or geographical characteristics.

Problem 1

The marks awarded for an assignment with respect to 20 students were as follows.

6	7	5	7	7	8	7	6	9	7
4	10	6	8	8	9	5	6	4	8

Represent the information in a frequency table.

Solution

Frequency Table

Mark	Tally	Frequency *(f)*
4	11	2
5	11	2
6	1111	4
7	~~1111~~	5
8	1111	4
9	11	2
10	1	1
Total		$\sum f = 20$

Problem 2

The number of mites collected from leaves in a garden were as follows.

28 122 217 130 120 85 80 90 120 140

70 40 146 167 113 90 58 174 194 170

100 75 104 97 75 122 100 84 109 120

81

Represent it in a frequency table

Solution

Frequency Table

Class interval	Tally	Frequency (*f*)
0 - 39	1	1
40 - 79	𝍸	5
80 - 119	𝍸 𝍸 11	12
120 - 159	𝍸 111	8
160 - 199	1111	4
200 - 239	1	1
		$\sum f = 31$

Problem 3

Calculate the following data and express the data using Pie diagram.

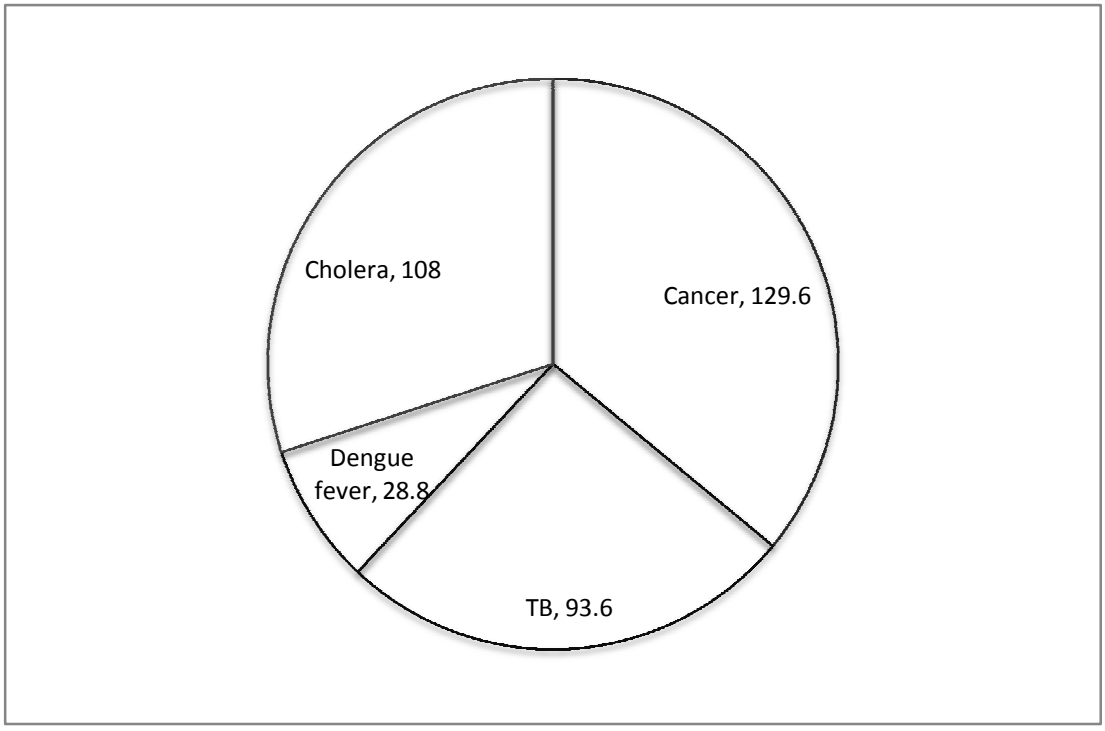
Annual mortality due to various diseases in a hospital.

Disease	Cancer	Tuberculosis	Dengue fever	Cholera
No. of death	18	13	4	15

Solution

Size of the angle = $\frac{Class\ frequency}{Total\ observation} \times 360^0$

Disease	No. of death	Frequencies out of 360	Proportional angles in degree
Cancer	18	$\frac{18}{50} \times 360$	129.6^0
TB	13	$\frac{13}{50} \times 360$	93.6^0
Dengue fever	4	$\frac{4}{50} \times 360$	28.8^0
Cholera	15	$\frac{15}{50} \times 360$	108^0
Total	50		360^0



Problem 4

Construct a simple bar diagram that represent plant diversity in a botanical garden.

Life forms	No. of species (frequency)
Herbs	310
Shrubs	236
Subshrubs	200
Trees	178
Climbers	112
Total	1036

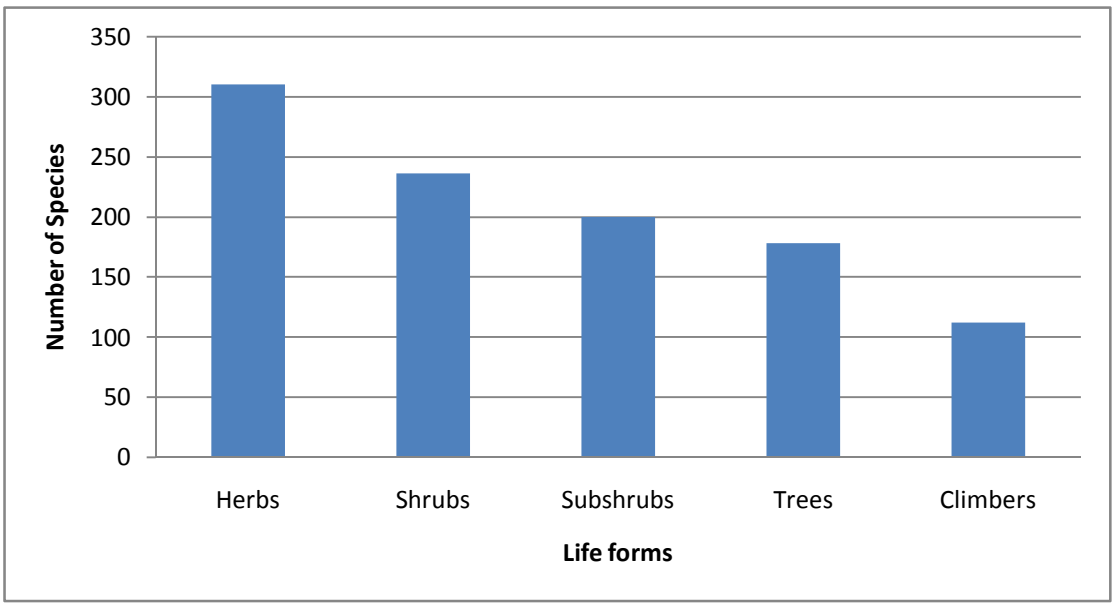
Solution

Step I.

Label the Y axis with dependant variable

Step II

Label the X axis with bar represents (discrete data)



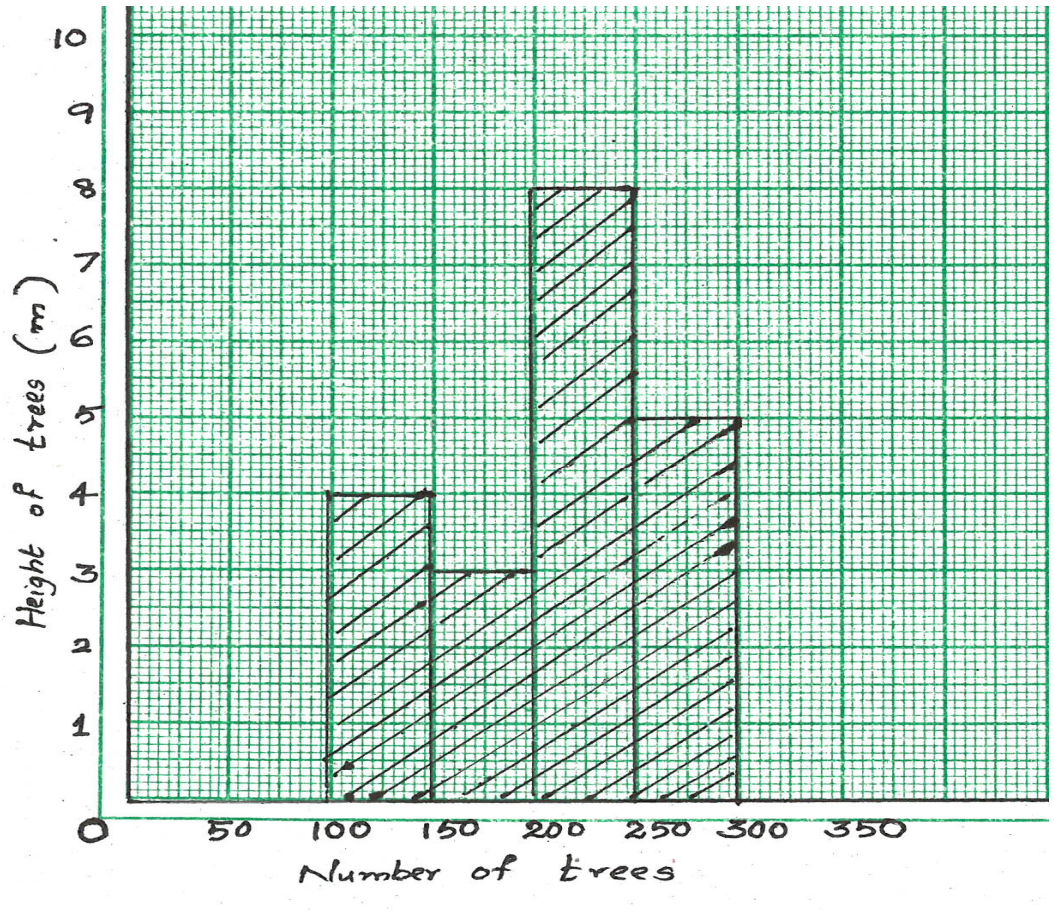
Problem 5

Construct a histogram using the following data. Height of trees in a forest ecosystem is as follows.

112, 124, 138, 148, 168, 188, 192, 202, 206, 212, 224, 232, 238, 242, 248, 252, 258, 282, 292, 296

Class interval (Height of trees in m)	No. of trees. (Frequency)
100 - 150	4
150 - 200	3
200 - 250	8
250 - 300	5
Total	$\sum f=20$

Solution



MEASURES OF CENTRAL TENDENCY

1. ARITHMETIC MEAN

Mean of a set of observation is their sum divided by the number of observations. It is the average of all observations. It is denoted by the symbol $\bar{x}$ (in sample) $\sum \frac{xi}{n}$ where 'n' denotes sample size

The population mean is given by the formula $\mu = \frac{\sum xi}{N}$. Where *x*i takes in all observation from xi to *xn*. Where *N* refers to number of observations

Problem 6

Calculate the arithmetic mean of plant height (m) from the following data.

Plant height	20-25	25-30	30-35	35-40	40-45	45-50	50-55
No. of plants	5	10	20	40	30	20	5

Solution

Class interval	Mid Value of Class interval (xi)	Frequency (fi)	Fixi
20-25	22.5	5	112.5
25-30	27.5	10	275
30-35	32.5	20	650
35-40	37.5	40	1500
40-45	42.5	30	1275
45-50	47.5	20	950
50-55	52.5	5	262.5
		$\sum fi = 130$	$\sum fixi$ =5025

$$\text{Arithmetic mean } \bar{x} = \frac{\sum fixi}{\sum fi}$$

$$= \frac{5025}{130}$$

$$= 38.65$$

Problem 7

Calculate the arithmetic mean from the following data

Class interval	10-20	20-30	30-40	40-50	50-60
Frequency	6	10	12	18	4

Solution

Class interval	Midvalue of class interval *(xi)*	Frequency *(fi)*	*fixi*
10-20	15	6	90
20-30	25	10	250
30-40	35	12	420
40-50	45	18	810
50-60	55	4	220
		N = 50	$\sum fixi = 1790$

Arithmetic mean $\bar{x} = \frac{\sum fixi}{N}$

where $\sum fixi$ = Sum of values of mid-point of class interval multiplied by their corresponding frequencies

N = Sum of frequencies

xi = $\frac{\text{Lower limit of class interval} + \textit{upper limit of class interval}}{2}$

$\bar{x} = \frac{1790}{50}$

$\bar{x} = 35.8$

2. MEDIAN

A median of a distribution is defined as the value of that variable which divides the total frequency into two equal parts when the series is arranged in ascending or descending order of magnitude.

Median when the number of observations (N) is odd is calculated by the equation:

$$\text{Median} = \frac{(N+1)^{th}}{2} \text{ item}$$

Median when the number of observations (N) is even is calculated by the equation:

$$\text{Median} = \frac{(N/2)^{th} + (N/2+1)^{th}}{2} \text{ item}$$

Median is the value when N refers to number of observation middle of a distribution. ie, half the scores are above the median and half are below the median. It is denoted by the symbol M

If 'N' (no . of observation) is odd number,

Then M = value of the $(\frac{N+1}{2})th$ observation

If 'N' is even number,

then M is equal to the mean of m^{th} and $m+1^{th}$ observation.

$$M = \frac{Value\ of\ m^{th}\ observation + Vaue\ of\ (m+1)^{th}\ observation}{2}$$

Problem 8

Find the median for the following data

Number of fishes	100	150	80	200	250	180
Number of ponds	24	26	16	20	6	30

Solution : Arrange the raw data in ascending order

Item No	Number of fishes	Number of ponds	Cumulative frequency
1	80	16	16
2	100	24	40
3	150	26	66
4	180	30	96
5	200	20	116
6	250	6	122

Number of items (N) = 6 (even), therefore

$$\text{Median} = \left(\frac{N+1}{2}\right)^{th} \text{ item } \frac{6}{2} + 1 = \frac{7}{2} = 3.5^{th} \text{ item}$$

Median lies between 3rd item (150) and 4th item (180)

$$\therefore Median = \frac{150 + 180}{2} = 165$$

Problem 9

Find the median for the following data obtained by counting the number of flowers on 19 plants.

Number of flowers	1	2	3	4	5
Number of plants	3	4	6	3	3

Solution

Arrange the data in ascending order

Item No.	Number of flowers	Number of plants	Cumulative frequency
1	1	3	3
2	2	4	7
3	3	6	13
4	4	3	16
5	5	3	19

Number of items N = 5 (odd)

$\therefore$ Median $= \frac{N+1}{2} = \frac{5}{2} + 1 = \frac{6}{2}$

$\therefore$ Median is 3rd item

$\therefore$Median M = 3

3. MODE

Mode is the number that occurs most frequently in a set of numbers. Mode is denoted by the symbol Z

Problem 10

Find the mode of the following data

Marks	1-5	6-10	11-15	16-20	21-25
Number of students	7	10	16	32	24

Solution

Marks (class interval)	Class boundary	Mid value of class interval	No. of students (Frequency)
1-5	0.5-5.5	3	7
6-10	5.5-10.5	8	10
11-15	10.5-15.5	13	16
16-20	15.5-20.5	18	32
21-25	20.5-25.5	23	24

Maximum frequency *(fm)* is 32 and it lies in the class boundary 15.5 – 20.5. Thus modal class is 15.5-20.5.

$$\text{Mode} = L1 + \frac{fm - f1}{2fm - f1 - f2} xi$$

Where L1 = Lower limit of modal class

fm = Frequency of modal class

f1 = Frequency of the preceding modal class

f2 = Frequency of the succeeding modal class

i = width of the class interval

L1 = 15.5 fm = 32 f1 = 16 f2 = 24 i = 5

$$\text{Mode Z} = 15.5 + \frac{32-16}{2x32-16-24} \times 5$$

$$= 15.5 + \frac{4}{6} \times 5$$

$$= 15.5 + 3.33$$

$$= 18.83$$

MEASURES OF DISPERSION

1. RANGE

Range of the raw data is the difference between the maximum and the minimum observations in the data.

R = H-L where, R = Range, H = Highest value of variable, L = Lowest value of variable

Problem 11

Find out the range to the following data

11, 21,22,10,9,29,33,14,14,15,18,45,32,29,40,21,44,52,39,49

Solution

In this data the highest observation is 52 and the lowest is 9

Range R = H-L where H= Highest value of variable and L = Lowest value of variable

Range R = 52-9

= 43

2. MEAN DEVIATION

Mean Deviation is the average of the absolute values of the deviation from the mean (or median or mode).

$$\text{Mean deviation MD} = \frac{\sum|xi-\bar{x}|}{n},$$

Problem 12

Find the mean deviation from the mean for the given raw data

12,6,7,3,15,10,18,5

Solution : Here a total of eight observations are given

$\therefore$ n = 8

Arithmetic mean $\bar{x} = \frac{12+6+7+3+15+10+18+5}{8}$

$= \frac{76}{8}$

$= 9.5$

Mean deviation MD = $\frac{\sum|xi-\bar{x}|}{n}$,

where xi = variable
$\bar{x}$ = *Mean*
n = Number of observations

Variable xi	Deviation (xi-$\bar{x}$)	Absolute value of deviation $\left\|xi-\bar{x}\right\|$
12	2.5	2.5
6	-3.5	3.5
7	-2.5	2.5
3	-6.5	6.5
15	5.5	5.5
10	0.5	0.5
18	8.5	8.5
5	-4.5	4.5
		$\sum\left\|xi-\bar{x}\right\| = 34$

Mean deviation MD or $\delta = {}^{34}\!/_{8}$ = 4.25

Problem 13

Calculate the mean deviation from the mean for the following frequency distribution

Mid value of class	15	20	25	30	35	40	45	50	55
Frequency	2	22	19	14	3	4	6	1	1

Solution

Midpoint xi	Frequency fi	$fixi$	Deviation (xi-$\bar{x}$)	Absolute value of deviation $\lvert xi-\bar{x}\rvert$	$fi\lvert xi-\bar{x}\rvert$
15	2	30	-12.85	12.85	25.7
20	22	440	-7.85	7.85	172.7
25	19	475	-2.85	2.85	54.15
30	14	420	2.15	2.15	30.1
35	3	105	7.15	7.15	21.45
40	4	160	12.15	12.15	48.6
45	6	270	17.15	17.15	102.9
50	1	50	22.15	22.15	22.15
55	1	55	27.15	27.15	27.15
	N = 72	$\sum fixi = 2005$			$\sum fi\lvert xi-\bar{x}\rvert = 504.9$

$$\text{Mean } \bar{x} = \frac{\sum fixi}{N} = \frac{2005}{72} = 27.85$$

Where fi = Frequency

xi = Midpoint of class

N = summation of frequency

$$\text{Mean deviation} = \sum \frac{f\lvert xi-\bar{x}\rvert}{N} = \frac{504.9}{72} = 7.0125$$

3. STANDARD DEVIATION

Standard deviation is the square root of the arithmetic mean of the squares of deviations from the arithmetic mean

Standard deviation $\sigma = \sqrt{\frac{1}{N}\sum fi(xi - \bar{x})^2}$

Where fi = Frequency,

xi = Mid-point of class interval

$\bar{x}$ = Arithmetic mean

N = Total number of observations

Problem 14

Find the standard deviation for the following frequency distribution

Class	Frequency *(f)*
0-10	5
10-20	7
20-30	10
30-40	6
40-50	2
	N= 30

Solution

Mid Value of class interval *xi*	Frequency *(fi)*	*fixi*	(xi-$\bar{x}$)	(xi- $\bar{x}$)^2	fi(xi- $\bar{x}$)^2
5	5	25	-17.67	312.23	1561.15
15	7	105	-7.67	58.83	411.81
25	10	250	2.33	15.43	54.30
35	6	210	12.33	152.03	912.18
45	2	90	22.33	498.63	997.26
	N=$\sum fi$ = 30	$\sum fixi$ = 680			$\sum fi(xi - \bar{x})^2$ = 3936.70

Mean $\bar{x}$=$\frac{1}{N}\sum fixi = \frac{680}{30} = 22.67$

Standard deviation $\sigma = \sqrt{\frac{1}{N}\sum fi(xi - \bar{x})^2}$

$= \sqrt{\frac{1}{30}}[3936.70]$

$= \sqrt{131.22} \quad = 11.46$

Problem 15

Calculate the standard deviation for the following data

7,8,11,6,13,8,10

Solution: Here Number of observations $n = 7$

Mean = $\frac{7+8+11+6+13+8+10}{7}$

$\bar{x} = 9$

Value (xi)	Deviation for mean $(xi - \bar{x})$	$(xi - \bar{x})^2$
6	-3	9
7	-2	4
8	-1	1
8	-1	1
10	1	1
11	2	4
13	4	16
		$\sum(xi - \bar{x})^2 = \mathbf{36}$

Standard Deviation $= \sqrt{\frac{\sum(xi-\bar{x})^2}{n}}$

$\sigma = \sqrt{\frac{36}{7}} = \sqrt{5.14}$

$= 2.27$

4. VARIANCE

Variance of a distribution is defined as the square of the Standard Deviation

$V = (S)^2$ where V = Variance, S = Standard Deviation

Problem 16

Haemoglobin content in g/100 ml of 10 persons of a locality was recorded as 7,8,9,10,11, 12,13,14,15 and 15.5. Find the variance of the data.

Solution : Variance or $(S^2) = \left[\frac{\sum fx^2}{\sum f} - \bar{x}^2\right]^2 =$ $V = \sum x^2 / N$

where x = variable X- mean $\overline{X}$

N = Number of observations

Following table is prepared to calculate variance

variable X Hb%	Deviation $X - \overline{X} = x$	x	x^2
7	7-11.5 = -4.5	-4.5	20.25
8	8-11.5 = -3.5	-3.5	12.25
9	9-11.5 = -2.5	-2.5	6.25
10	10-11.5 = -1.5	-1.5	2.25
11	11-11.5= -0.5	-0.5	0.25
12	12-11.5=0.5	0.5	0.25
13	13-11.5=1.5	1.5	2.25
14	14-11.5 = 3.0	3.0	9.0
15	15-11.5 = 3.5	3.5	12.0
15.5	15.5-11.5=4	4	16.0
$\sum X = 115$			$\sum x^2 = 81$

Arithmetic mean $\overline{X} = \frac{\sum X}{N}$ where

$\sum X$ = summation of variables

N = Number of observations

$\sum x^2$ = summation of square of deviation

$\sum X = 115$ $\overline{X} = 115/10 = 11.5$

$\therefore \sum x^2 = 81$

Variance $= \frac{\sum x^2}{N}$

$= \frac{81}{10}$

Variance V = 8.1

5. COEFFICIENT OF VARIATION

The Standard Deviation is an absolute measure of dispersion. The corresponding relative measure is known as the Co-efficient of Variance.

C.V. = S/ X x 100 where CV = Co-efficient of Variance, S = Standard Deviation, X = Arithmetic Mean

Problem 17

An analysis of seed number per fruit in 10 fruits each of two batches is given below. Find coefficient of variation of both batch and mention which of the two groups has lower range of variance

Solution

Fruit No	No. of seeds Batch I X_1	No. of seeds Batch II X_2
1	7	10
2	9	8
3	6	9
4	8	10
5	6	11
6	5	10
7	7	5
8	8	6
9	6	4
10	8	7
	$\sum x_1 = 70$	$\sum x_2$=80

Solution : Here $N_1 = 10$ and $N_2 = 10; \sum x_1 = 70$ *and* $\sum x_2 = 80$

Mean of Batch 1, $\bar{x_1} = \frac{70}{10} = 7$

Mean of Batch 2, $\overline{x_2} = \frac{80}{10} = 8;$

SD of Batch I where $\bar{x}$ = 7

Variable *x*	Deviation $x - \bar{x}$	$(x - \bar{x})^2$
7	0	0
9	2	4
6	-1	1
8	1	1
6	-1	1
5	-2	4
7	0	0

8	1	1
6	-1	1
8	1	1
		$\sum(x-\bar{x})^2$=14

Variance = $\frac{\sum(x-\bar{x})^2}{N-1}$ where N is the number of observations

$= \frac{14}{10-1} = \frac{14}{9} = 1.6$

(S_1) SD = $\sqrt{1.6} = 1.26$

SD of Batch II where $\bar{x}$ = 8

Variable *x*	Deviation $x-\bar{x}$	$(x-\bar{x})^2$
10	2	4
8	0	0
9	1	1
10	-2	4
11	-3	9
10	2	4
5	-3	9
6	-2	4
4	-4	16
7	-1	1
		$\sum(x-\bar{x})^2$=52

Variance = $\sum\frac{(x-\bar{x})^2}{N-1}$

Variance = $\frac{52}{10-1} = \frac{52}{9} = 5.78$

(S_2) SD = $\sqrt{5.78} = 2.4$

Coefficient of variation = $\frac{s}{\bar{x}}$ x 100

CV_1 = $\frac{1.26}{7}$ x 100 = 18%

CV_2 = $\frac{2.4}{8}$ x 100 = 30%

Conclusion : Batch I with lesser value of CV (18%) is more consistent in producing seeds than Batch II with CV 30%

Problem 18

Following are the weight of two rats in 10 months. Both were fed same normal diet. If the consistency performance is the criterion, Calculate which one maintain consistency?

Rat X	50	45	55	40	47	50	45	40	48	50	$\sum x = 470$
Rat Y	55	46	51	45	52	45	50	45	40	47	$\sum Y = 476$

Solution - Frame a table to obtain various values to calculate CV.

No.	Variable x	Deviation $(x - \bar{x})$	$(x - \bar{x})^2$	Variable y	Deviation $(y - \bar{y})$	$(y - \bar{y})^2$
1	50	+3	9	55	+7.4	54.76
2	45	-2	4	46	-1.6	2.56
3	55	+8	64	51	+3.4	11.56
4	40	-7	49	45	-2.6	6.76
5	47	0	0	52	+4.4	19.36
6	50	+3	9	45	-2.6	6.76
7	45	-2	4	50	+2.4	5.76
8	40	-7	49	45	-2.6	6.76
9	48	+1	1	40	-7.6	57.76
10	50	+3	9	47	-0.6	0.36
	$\sum x = 470$	$=\sum x = 0$	$\sum(x - \bar{x})^2 = 90$	$\sum y = 476$	$\sum y = 0.6$	$\sum(y - \bar{y})^2 = 172.4$

CV for the x rat,

Mean $\bar{x}$ = $\frac{470}{10} = 47$

$\bar{x}$ = $\frac{\sum x}{N} = \frac{470}{10} = 47$

S.D = $\sqrt{\frac{\sum x^2}{N}} = \sqrt{\frac{198}{10}} = \sqrt{19.8} = 4.44$

CV = $\frac{S.D}{\bar{x}}$ x 100 = $\frac{4.44}{47}$ x 100 = 9.44

Co efficient of variation of the Rat x = 9.44

CV for the y Rat.

Mean $\bar{y}$ = $\frac{\sum y}{N} = \frac{476}{10} = 47.6$

S.D = $\sqrt{\frac{\sum y^2}{N}} = \sqrt{\frac{172.40}{10}} = 4.15$

CV = $\frac{S.D}{\bar{y}}$ x 100 = $\frac{4.15}{47.6}$ x 100 = 8.71

Coefficient of variation of Y Rat = 8.71

Conclusion : Since Co-efficient of variation is less (8.71), in the case of Y rat as compared to X Rat (9.44),Y is more consistent.

TEST OF SIGNIFICANCE

CHI - SQUARE TEST

Chi- Square test is the test of significance of overall deviation square in the Observed frequencies and Expected frequencies divided by Expected frequencies.

$$X^2 = \sum \frac{(observed-expected)^2}{expected}$$

$$= \sum \frac{(O-E)^2}{E}$$

The value of X^2 will be zero if O = E in each class. The observed results are based on the number of degree of freedom (df) which is equal to the number of classes (k) minus one and the critical level of probability (5% or 1%). The expected value of X^2 is obtained from the Chi-square Distribution table and compared with the observed value. If the table value is less than the calculated value, then the difference between the Observed values and Expected values is significant.

A Chi Square Test is used to test whether frequency distribution obtained experimentally fit an expected frequency distribution that is based on the theoretical or previously known probability of each outcome.

An experiment is conducted by taking a simple random sample from a population, and each member of the population is grouped into exactly one of categories.

Step 1 : The observed frequencies are calculated for sample.

Step II : The expected frequences are obtained from previous knowledge (or brief) or probability theory. In order to proceed to the next step, it is necessary that each expected frequency is at least 5.

Step III : A hypothesis is formulated :

(i) The null hypothesis H_O : the population frequencies are equal to the expected frequencies.

(ii) The alternative hypothesis H_a : The null hypothesis is false (i.e, Population frequencies is not equal to expected frequencies)

(iii) The degree of freedom : k - 1

(iv) α is the level of significance.

(v) A test statistic is calculated : $X^2 = \sum \frac{(observed-expected)^2}{expected}$

$= \sum \frac{(O-E)^2}{E}$

(vi) From α and k - 1; a critical value is determined from X^2 table.

(vii) Reject H_O if X^2 is larger than critical value (calculated value) and accept the Alternative Hypothesis

PROBLEM 19

In a cross between TT and tt , 1572 tall and 554 dwarf plants were obtained. Find out whether the segregation follows Mendelian principles

(Table value of X^2 for 1 degree of freedom at 5% level of significance is 3.84)*

Solution : Null Hypothesis Ho is that segregation follows Mendelian principles. According to the Mendel's Law of segregation phenotypic ratio in Monohybrid cross is 3:1 or $^3/_4 : ^1/_4$. Therefore,

Expected no. of TT = Total No. $\times \frac{3}{4}$

= (1572 + 554) $\times \frac{3}{4}$

= 2126 $\times \frac{3}{4}$

= 1594.5

Expected no. of tt = Total No $\times \frac{1}{4}$

= 2126 $\times \frac{1}{4}$

= 531.50

Phenotype	TT	tt
Observed value (O)	1572	554
Expected value (E)	1594.5	531.5
O-E	-22.5	22.5
$\frac{(O-E)^2}{E}$	$\frac{(-22.5)^2}{1594.5}$	$\frac{(22.5)^2}{531.5}$
	0.317	0.952

$$X^2 = \sum \frac{(O-E)^2}{E}$$

$$= 0.317 + 0.952$$

$$= 1.26$$

Since the calculated value of X^2 is less than the table value 3.84, the test is insignificant. Therefore, the test follows Null Hypothesis that is segregation follows Mendelian Principle.

Problem 20

With the help of suitable statistical tests, check whether the given F2 data is in accordance with Mendelian Law of Independence Assortment

Class	Frequency
Red flower and Long leaves	44
Red flower and broad leaves	17
White flower and long leaves	13
White flower and broad leaves	6
Total	80

(Table value of X^2 for 3 degrees of freedom at 5% level is 7.81)*

Solution : Null Hypothesis Ho is the data is in accordance with Mendelian law of Independent Assortment. Let Red flower be represented as RR and White flower as rr. Let long leaves be represented as LL and broad leaves as ll. Then, according to Mendel's Law of Independent Assortment, the phenotypic ratio of Dihybrid cross is 9:3:3:1 or $\frac{9}{16}:\frac{3}{16}:\frac{3}{16}:\frac{1}{16}$

Therefore Expected frequency of phenotype

RRLL = Total No. of F_2 offsprings $\times \frac{9}{16}$

= $80 \times \frac{9}{16} = 45$

RRll = $80 \times \frac{3}{16} = 15$

rrLL = $80 \times \frac{3}{16} = 15$

rrll = $80 \times \frac{1}{16} = 5$

Phenotype	RRLL	RRll	rrLL	rrll
Observed freq (O)	44	17	13	6
Expected freq (E)	45	15	15	5
O-E	-1	2	-2	1
$\frac{(O-E)^2}{E}$	0.02	0.26	0.26	0.2

$X^2 = \sum \frac{(O-E)^2}{E}$

= 0.02+0.26 +0.26+0.2

X^2 = 0.74

Since calculated value of X^2 is less than Table value 7.81, test is insignificant. ∴ F_2 data is in accordance with Mendelian Law of Independent Assortment.

* Refer Chi- Square Distribution Table.

Chi-square Distribution Table

d.f.	.995	.99	.975	.95	.9	.1	.05	.025	.01
1	0.00	0.00	0.00	0.00	0.02	2.71	3.84	5.02	6.63
2	0.01	0.02	0.05	0.10	0.21	4.61	5.99	7.38	9.21
3	0.07	0.11	0.22	0.35	0.58	6.25	7.81	9.35	11.34
4	0.21	0.30	0.48	0.71	1.06	7.78	9.49	11.14	13.28
5	0.41	0.55	0.83	1.15	1.61	9.24	11.07	12.83	15.09
6	0.68	0.87	1.24	1.64	2.20	10.64	12.59	14.45	16.81
7	0.99	1.24	1.69	2.17	2.83	12.02	14.07	16.01	18.48
8	1.34	1.65	2.18	2.73	3.49	13.36	15.51	17.53	20.09
9	1.73	2.09	2.70	3.33	4.17	14.68	16.92	19.02	21.67
10	2.16	2.56	3.25	3.94	4.87	15.99	18.31	20.48	23.21
11	2.60	3.05	3.82	4.57	5.58	17.28	19.68	21.92	24.72
12	3.07	3.57	4.40	5.23	6.30	18.55	21.03	23.34	26.22
13	3.57	4.11	5.01	5.89	7.04	19.81	22.36	24.74	27.69
14	4.07	4.66	5.63	6.57	7.79	21.06	23.68	26.12	29.14
15	4.60	5.23	6.26	7.26	8.55	22.31	25.00	27.49	30.58
16	5.14	5.81	6.91	7.96	9.31	23.54	26.30	28.85	32.00
17	5.70	6.41	7.56	8.67	10.09	24.77	27.59	30.19	33.41
18	6.26	7.01	8.23	9.39	10.86	25.99	28.87	31.53	34.81
19	6.84	7.63	8.91	10.12	11.65	27.20	30.14	32.85	36.19
20	7.43	8.26	9.59	10.85	12.44	28.41	31.41	34.17	37.57
22	8.64	9.54	10.98	12.34	14.04	30.81	33.92	36.78	40.29
24	9.89	10.86	12.40	13.85	15.66	33.20	36.42	39.36	42.98
26	11.16	12.20	13.84	15.38	17.29	35.56	38.89	41.92	45.64
28	12.46	13.56	15.31	16.93	18.94	37.92	41.34	44.46	48.28
30	13.79	14.95	16.79	18.49	20.60	40.26	43.77	46.98	50.89
32	15.13	16.36	18.29	20.07	22.27	42.58	46.19	49.48	53.49
34	16.50	17.79	19.81	21.66	23.95	44.90	48.60	51.97	56.06
38	19.29	20.69	22.88	24.88	27.34	49.51	53.38	56.90	61.16
42	22.14	23.65	26.00	28.14	30.77	54.09	58.12	61.78	66.21
46	25.04	26.66	29.16	31.44	34.22	58.64	62.83	66.62	71.20
50	27.99	29.71	32.36	34.76	37.69	63.17	67.50	71.42	76.15
55	31.73	33.57	36.40	38.96	42.06	68.80	73.31	77.38	82.29
60	35.53	37.48	40.48	43.19	46.46	74.40	79.08	83.30	88.38
65	39.38	41.44	44.60	47.45	50.88	79.97	84.82	89.18	94.42
70	43.28	45.44	48.76	51.74	55.33	85.53	90.53	95.02	100.43
75	47.21	49.48	52.94	56.05	59.79	91.06	96.22	100.84	106.39
80	51.17	53.54	57.15	60.39	64.28	96.58	101.88	106.63	112.33
85	55.17	57.63	61.39	64.75	68.78	102.08	107.52	112.39	118.24
90	59.20	61.75	65.65	69.13	73.29	107.57	113.15	118.14	124.12
95	63.25	65.90	69.92	73.52	77.82	113.04	118.75	123.86	129.97
100	67.33	70.06	74.22	77.93	82.36	118.50	124.34	129.56	135.81

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MICROTECHNIQUE

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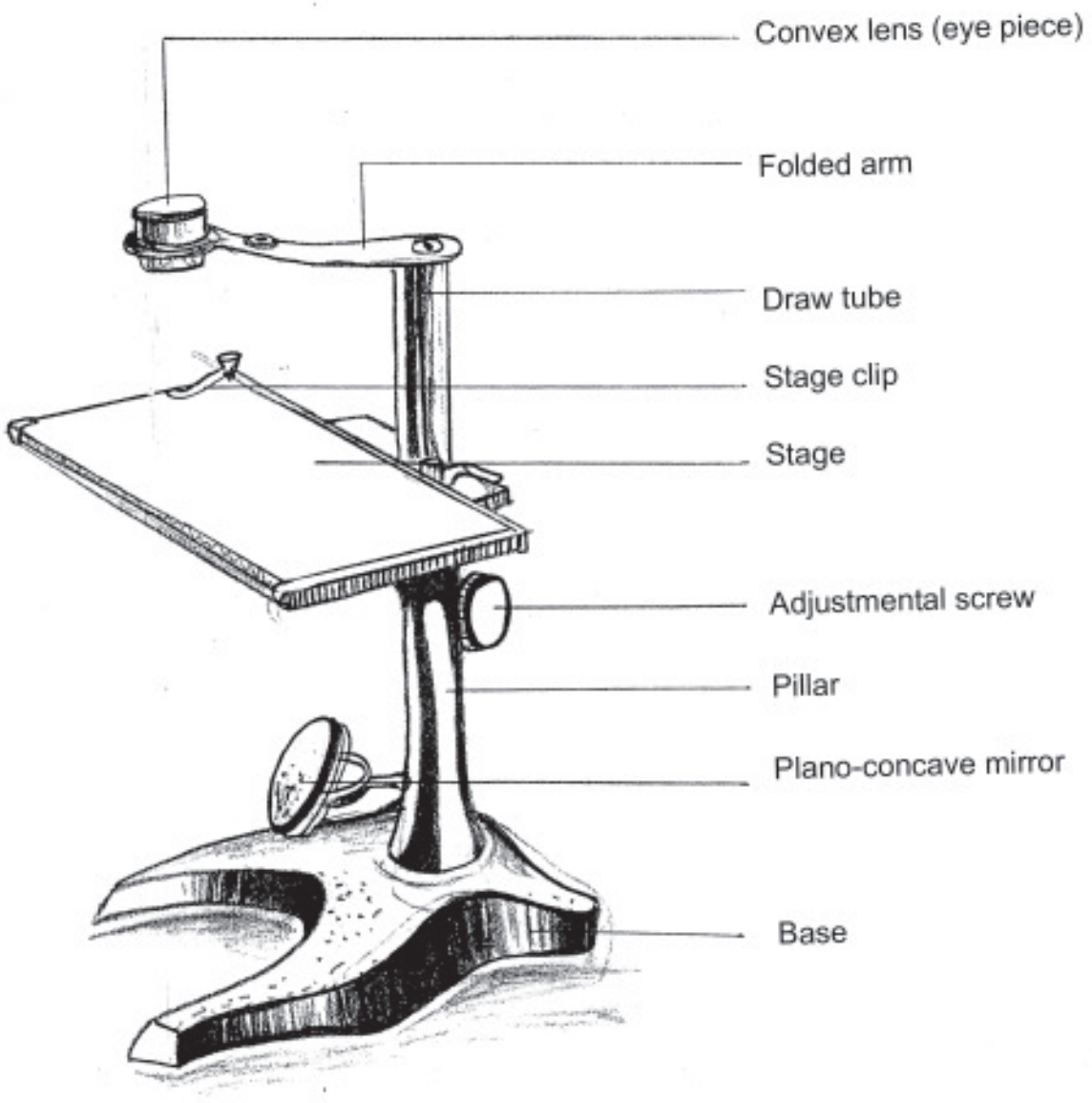
SIMPLE MICROSCOPE

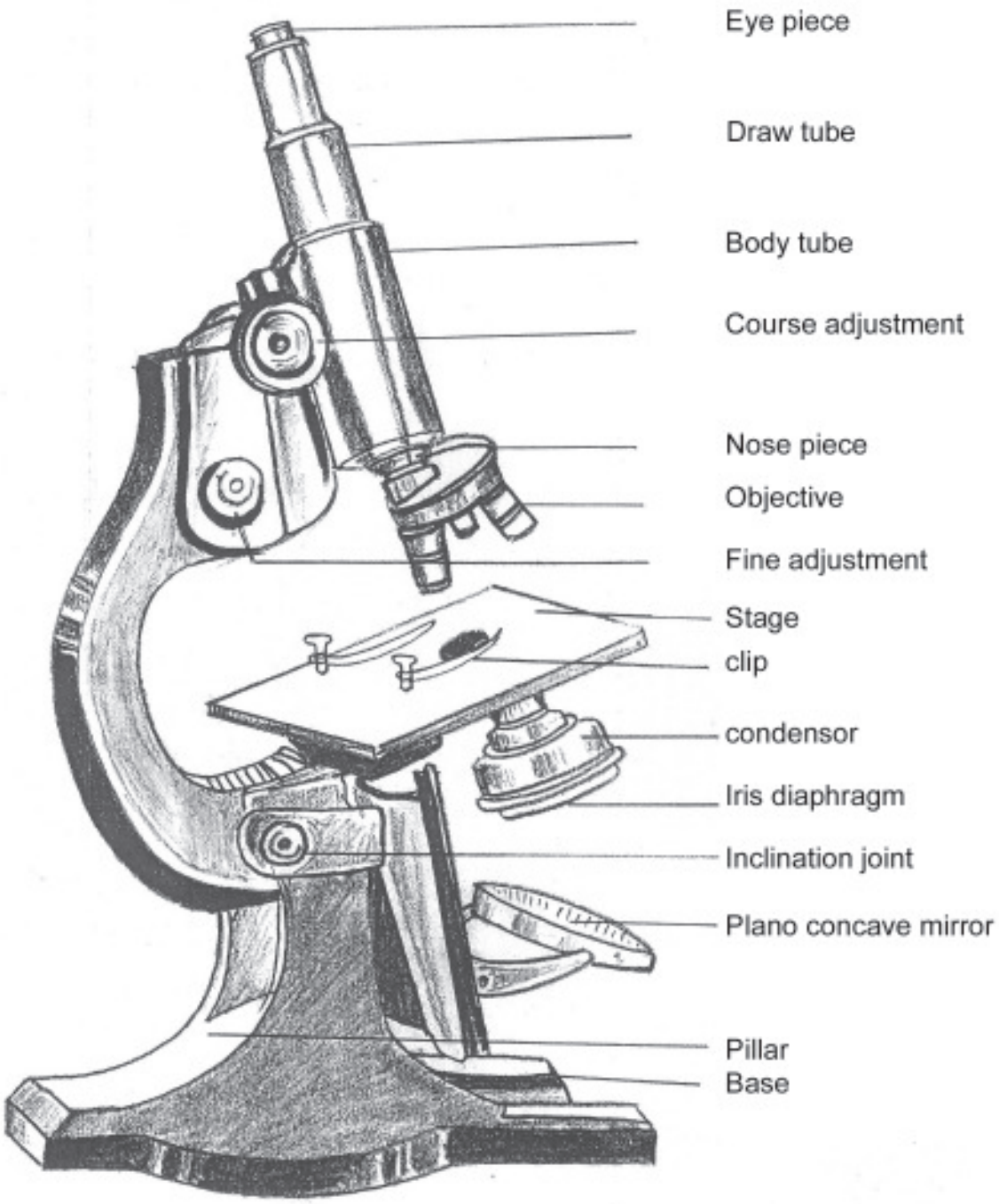


SIMPLE MICROSCOPE

Dissection microscope is a simple brightfield light microscope. It is used for magnifying the object for morphological and anatomical studies. It has only a single lens system. The magnification produced is 10X. It produces a virtual, erect, magnified image of the object. The different parts of a dissection microscope are:

i. Base or foot
ii. Pillar (Stand or limb)
iii. Draw tube
iv. Stage or Platform
v. Stage clips
vi. Folded arm
vii. An eyepiece with convex lens
viii. Plano-concave mirror

COMPOUND MICROSCOPE



COMPOUND MICROSCOPE

Compound microscope is a bright field light microscope used for magnification using visible light (wavelength 380 – 740 nm) as the source of illumination. It has both mechanical as well as optical components. It has two optical components each of which acts as a single positive lens.The magnifications produced are 100x (low power) and 400x - 450x (high power) The different parts are:

I Mechanical components

1. Base (foot / stand)
2. Pillar
3. Arm / limb
4. Inclination joint or swivel
5. Body tube
6. Nose piece
7. Draw tube
8. Stage or platform
9. Stage clips
10. Sub-stage
11. Iris diaphragm
12. Coarse adjustment
13. Fine adjustment

II Optical components

14. Plano concave mirror
15. Condensor lens
16. Objective lens
17. Ocular lens/ Eye piece

FIXATIVES

Fixation is the process of preservation of all cellular and structural elements as nearly as the natural living condition as possible. In Chemical fixation, a single fixing fluid or a combination of reagents are used as fixatives.

1. Formalin – Acetic acid –Alcohol

This fixative mixture is commonly known as FAA. It is a coagulant widely used to study anatomical and morphological aspects. It is unsuitable for chromosome studies. Specimens are kept indefinitely in FAA without any damage. The minimum time required for fixation is 18 hours. Later specimens are transferred to 70% ethyl alcohol for permanent preservation. FAA gives acid fixation image.

Composition

Ethyl alcohol (95%, 75% or 50%)	:	90 ml
Glacial Acetic acid	:	5 ml
Formalin	:	5 ml

2. Carnoy's Fluids

This fixative mixture is commonly used for cytological studies. The root tips are kept for 15 minutes and anthers for an hour in this fixative. Fixed material should be washed twice with 95% ethyl alcohol before further processing. It penetrates rapidly into tissues. These are of two types:

Type 1 : Farmer's formula

Composition

Absolute ethyl alcohol	:	15 ml
Glacial Acetic acid	:	5 ml

Type 2 : Carnoy's formula

Composition

Absolute ethyl alcohol	:	30 ml
Glacial Acetic acid	:	5 ml
Chloroform	:	15 ml

STAINS

A stain is a chemically defined substance used to colour biological specimens. The presence of colour in a stain is due to the presence of groups or radicals called chromophores. The power of imparting a colour to other substances is due to the presence of an Auxochrome. Auxochromes are either acid or alkali radicals which are also responsible for the solubility of stains.

1. Safranin

Safranin is a synthetic stain obtained from Coal-Tar. It is basic in nature with chromophore as cation. It has greater combining capacity at alkaline pH. It stains acidic substances such as nucleus, chromosomes and lignin. It is a good combination with fast green in double staining technique.

Composition

Safranin O	:	2.25 g
Alcohol 95%	:	225 ml

Dissolve the stain in alcohol and dilute to required concentration with distilled water.

2. Acetocarmine

Acetocarmine is a natural stain of animal origin. It stain chromosomes. Cochineal is a yellowish –red powder obtained from female cochineal insect *Coccus cacti*. Carmine is bright red in colour and is obtained by adding Alum to Cochineal.

Preparation

Boil 100 ml of 45% acetic acid in a flask fitted with a reflux condensor. Add a small quantity of carmine powder from the weighed out quantity of 2g. Remove the source of heat and add the rest of the dye, dissolve, cool and filter. Add a few drops of ferric acetate dissolved in glacial acetic acid until the colour is wine-red. Store in a refrigerator.

3. Hematoxylin

Hematoxylin is a natural stain of plant origin. It is obtained from heartwood of *Hematoxylon campechianum* (log wood). It is oxidised by atmospheric oxygen to form reddish dye hematein. Hematein forms complexes (chelates) with complex cations formed when salts of iron or aluminium (mordants) are dissolved in water. Therefore, stain is prepared with salts like iron, aluminium and copper.

DEHYDRATING AGENTS

Dehydration is a slow process of removal of water from the fixed and hardened tissues. It helps to preserve the material for long storage. The process of dehydration consists of treating the tissues with a series of solutions containing progressively increasing concentrations of the dehydrating agent and decreasing concentration of water.

1. Ethanol

Ethanol is the most commonly used dehydrating agent. Tissues are passed through ethanol- water mixture with increase in concentration of ethanol and decrease in concentration of water in each series (5% to 100%). Time in each grade is 30 minutes for small materials and 12 hours for big specimens. Ethyl alcohol is a non-solvent of paraffin. It may cause excessive hardening and shrinkage of tissues, if fixed schedule is not suited to the tissue.

2. Glycerin

Glycerin is a dehydrant used for algae and other delicate objects. The slow, progressive dehydration prevents sudden changes in concentration and minimises plasmolysis. The high boiling point of glycerine permits the elimination of water by evaporation.

3. Tertiary butyl alcohol

TBA is a dehydrant and a solvent of paraffin. Tissues washed in water are dehydrated in ethyl alcohol up to 50% and then transferred to TBA series from 50% onwards. The tissues are kept in each series up to 2 to 3 hours until it reaches pure TBA series.

MOUNTING MEDIA

A mounting media preserve the sections in a sufficiently transparent condition for microscopic investigation. A good mounting medium should have the following characteristics:

1. It should have the correct refractive index, should be transparent, should not turn yellow or any other colour when exposed to light or when stored for long time.
2. It can be dissolved in solvents such as xylene or toluene and should not take too much time to harden.
3. It should possess good adhesive property to the glass slide.
4. It should be free from acidity.
5. It should not have too low melting point.

1. DPX

Destrine Plasticiser Xylene (DPX) is a synthetic resin used as mounting medium. The DPX mixture is completely colourless. It preserves the colour of basic dyes and also the medium. It provides clear visibility. The pH of the mixture does not change upon long storage.

Preparation

Mix 7.5ml of tricresylphosphate with 40ml of xylene and add 10g of distrene.

2. Canada Balsam

Canada Balsam is a natural resin used as mounting medium. It is extracted from the bark of *Abies balsamea*. It is a viscid yellowish or greenish substance soluble in xylene, dioxane or trichloroethylene. Benzene, Chloroform and ethanol are used as solvents of Canada Balsam.

SMEAR

Smear is a technique used in Cytological studies of soft tissues that are not firmly united to one another by middle lamellae. In this method, the cells are directly spread uniformly on a clean slide with fixation or without pre-treatment to attain cell separation. The advantage of this method is that it avoids the lengthy process of dehydration, embedding, sectioning etc. No adhesives are required for affixing the cells. For permanent preparation, the cells may be killed, fixed, dehydrated and stained properly. This technique is is a very quick and simple method for studying mitosis and meiosis. The two commonly used methods are the Acetocarmine method and Feulgen method.

1. Acetocarmine Smear method

Fresh anther is smeared in a drop of Acetocarmine stain on a clean glass slide. It is dissected under a dissection microscope discarding pieces of anther wall and leaving masses of sporocytes. A cover glass is placed over the drop of the dye containing sporocytes and pressed gently. Fixed the sporocytes by heating the slide over a low flame. Drained off excess stain. The edges of the cover glass are sealed with paraffin and examined under microscope for stages of meiosis. Root tip smears are used for chromosome count and their morphological studies.

2. The Feulgen method

This method is also used to study DNA using Feulgen reagent as the procedure followed above. Lillie described an error – proof method of making the Shiff reagent for the Feulgen stain.

SQUASH

The technique of squashing is adopted for cytological studies of materials that are neither too soft to make a smear nor too hard to be sectioned. In this method the cells are separated by removing the pectic cementing materials of the middle lamella. It is effected by two methods:

1. Chemical hydrolysis
2. Enzyme hydrolysis

1. Chemical hydrolysis

The widely used chemical agent for softening the tissue is diluted hydrochloric acid (1N) followed by gentle warming. It is also achieved by using a mixture consisting of equal parts of 95% alcohol and concentrated HCl as fixative. No warming is needed and the tissue becomes soft within 5 minutes.

2. Enzyme hydrolysis

Mostly enzymes employed in dissolution of middle lamella are pectinases.

In both treatments, the softened material is washed with water thoroughly and is placed on a clean glass and covered with a coverslip and testing whether it disintegrates or not for uniform spreading by gentle tapping. They are stained, excess stain removed and fixed by gentle warming. It can be made permanent by dehydration followed by mounting.

MACERATION

Maceration is a technique for cytological studies of three – dimensional nature of structure elements of hard tissues. Isolation of cells from tissues is accomplished by chemical and mechanical means. For this the material is sliced and treated with any one of the following maceration processes. There are four methods of maceration.

1. Schultze's method

The material is covered with concentrated nitric acid in a test tube and a few crystals of potassium chlorate added. It is heated on a sand bath in a closed hood until the material is bleached white. The pulp is washed thoroughly by decanting process. It is shaken with glass beads until the material disintegrates.

2. Jeffrey's method

The macerating fluid consists of equal volumes of 10% chromic acid and 10% nitric acid. Treat for 1 to 2 days at 30 to 40 ºC, wash and shake with glass beads for disintegration of tissues.

3. Harlow's method

Treat the boiled material in chlorine water for 2 hours. Wash in water. Boil in 3% sodium sulphite for 15 minutes. Wash and macerate.

4. Acidic ethanol/ Ammonium oxalate method

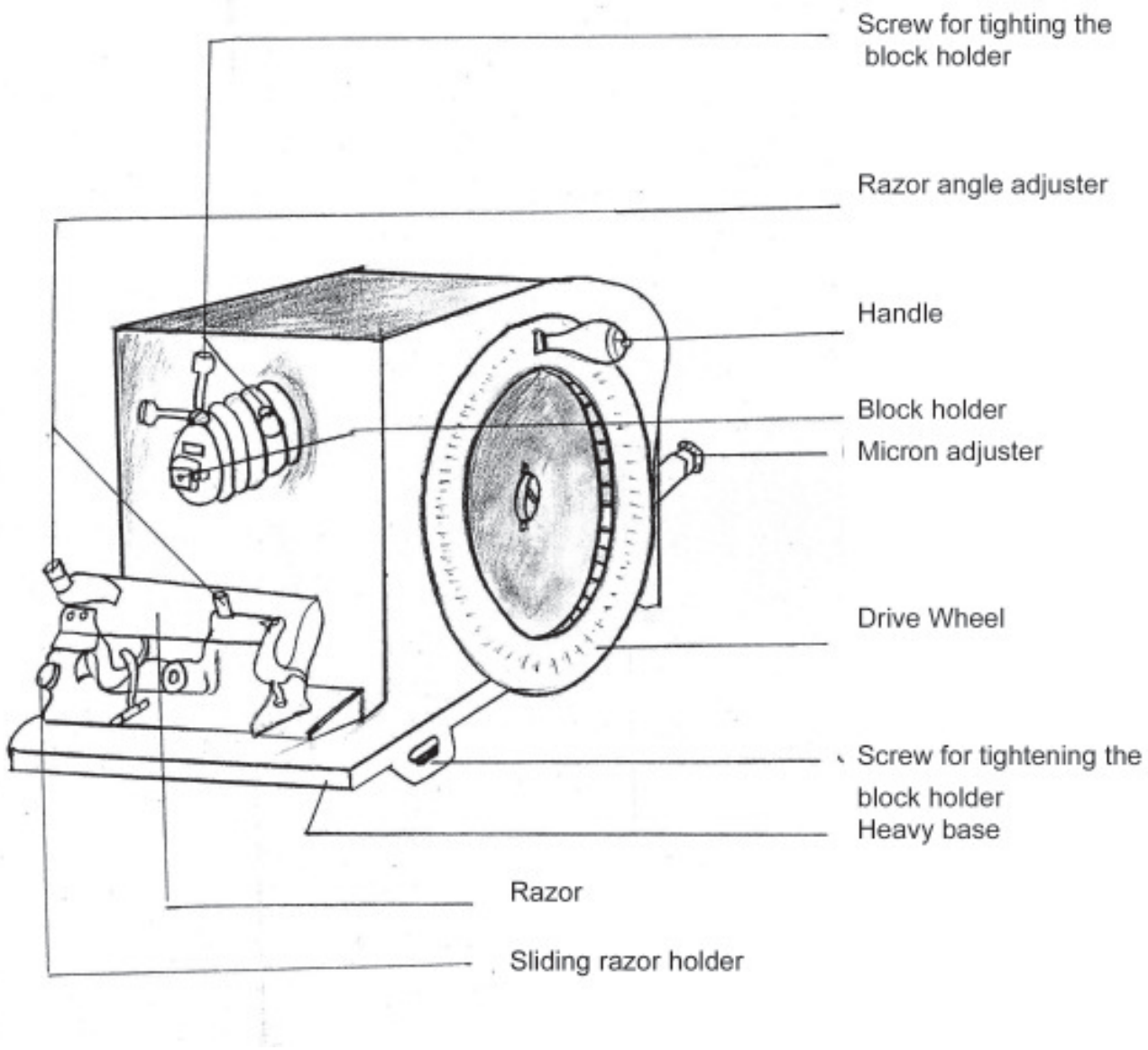
Tissues are vacuum infiltrated in a mixture of concentrated HCl and 70% ethanol (1:3 in water). Later transfer to fresh mixture and allow to stand for 24 hours at room temperature. Rinse thoroughly and transfer to 0.5% ammonium oxalate.

PHOTOMICROGRAPHY

Photomicrography refers to photography through a compound microscope using digital camera. The camera is linked to a computer system and a non- removable zoom – lens. The illumination includes the type of light source, Collector lenses, Condenser and Correction filters. There are three different methods of taking pictures.

1. Using a protected eyepiece, the lens of the camera is directly placed on the ocular. By manual focusing, images are taken.
2. Using an adaptor camera is mounted on eyepiece to correct lateral chromatic aberrations.
3. The camera is mounted on a phototube. It offers stable configuration and helps to avoid vibration.

ROTARY MICROTOME



MICROTOME

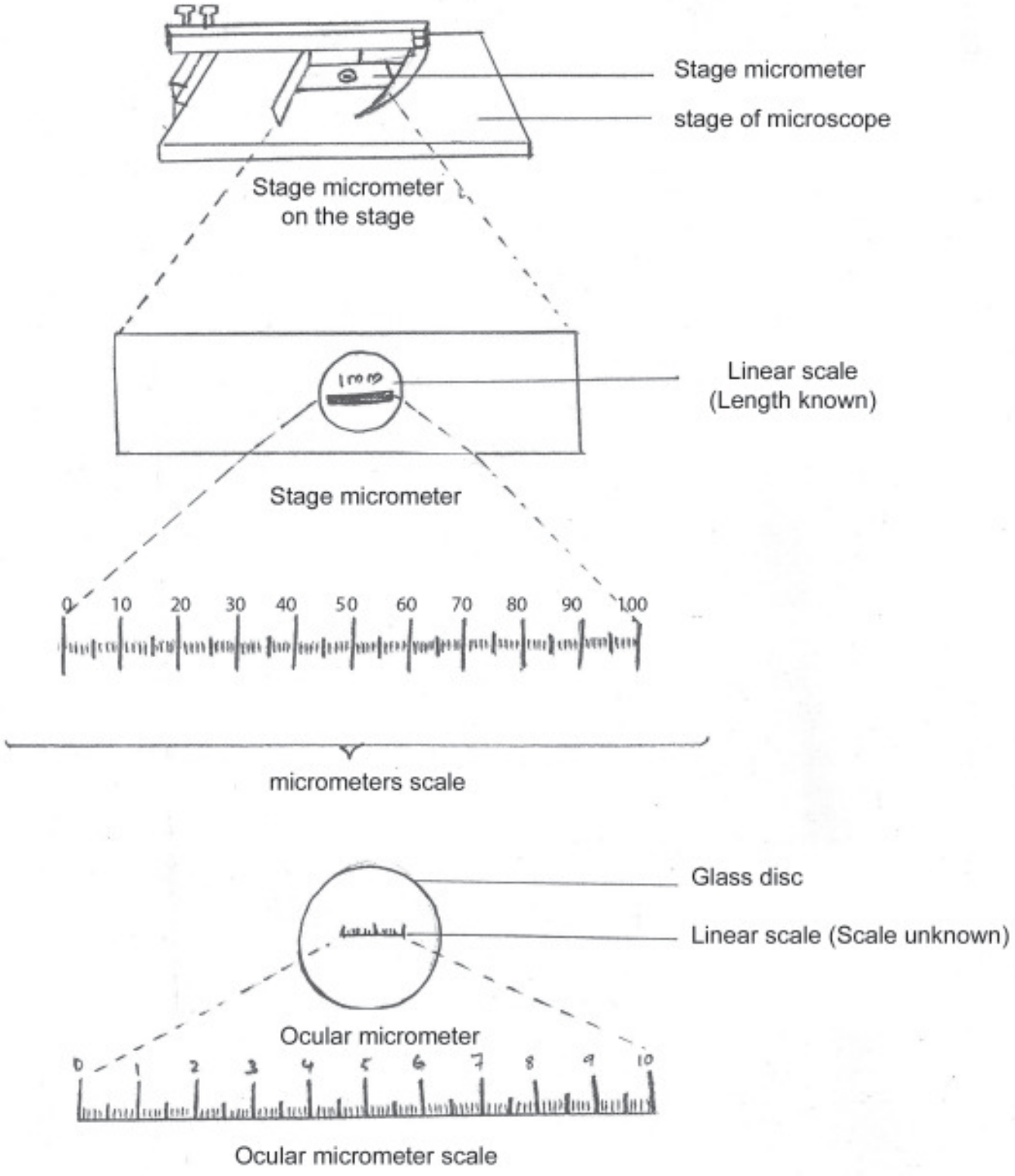
Microtome is the mechanical device or instrument used for cutting thin sections with thickness in micrometer (μm) of objects for microscopic observation. It contains three components.

1. A cutting knife
2. A Block holder
3. A knife holder

ROTARY MICROTOME

The cutting knife is stationary and block holder with material moves over the blade by turning the drive wheel. Sections of desired thickness of material are produced by adjusting a rotating circular disc called advance wheel located at the rear end. The knife –holder can be suitably adjusted through a wide range of cutting angles for better results.

MICROMETRY


Stage micrometer
stage of microscope
Stage micrometer on the stage
Linear scale (Length known)
Stage micrometer
0 10 20 30 40 50 60 70 80 90 100
micrometers scale
Glass disc
Linear scale (Scale unknown)
Ocular micrometer
0 1 2 3 4 5 6 7 8 9 10
Ocular micrometer scale

MICROMETRY

Micrometry is the measurement in micrometres (μm) of different dimensions of microscopic objects using a microscope. The device used for measuring microscopic objects is called micrometer. A micrometer has two components namely the Stage micrometer and the Ocular micrometer. Stage micrometer is a rectangular glass slide with 1 mm long scale with 100 equal divisions, each division with 0.01mm or 10 μm. Ocular micrometer is a transparent circular glass disc place inside the ocular tube. The scale has 100 equal divisions. The value of each division is unknown. It is calibrated with known scale of the stage micrometer using the formula

$V = X/Y \times 10$

V = Value of one ocular division
X= No. Of counted divisions on stage micrometer
Y = No. Of counted divisions on the ocular micrometer

The actual length of a microscopic object can be obtained by multiplying the number of ocular divisions overlapped by the object with the calibrated value.

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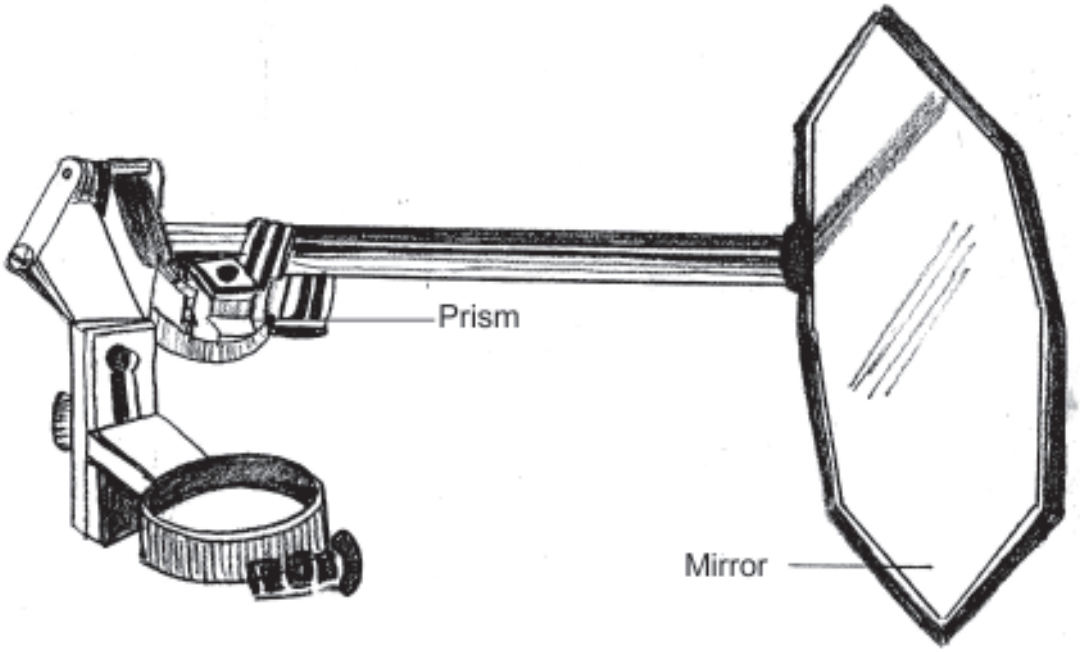
MIRROR – TYPE CAMERA LUCIDA



CAMERA LUCIDA

Camera lucida is an accessory instrument, mounted on the top of the body tube of a compound microscope. It is used for making accurate, clear, simple and exactly proportionate outline sketches of the objects under study. It produces an optical superimposition of the object and the surface on which figures are drawn. The magnification of the diagram depends upon the magnification of the combination of eye piece and the objective lens used. It was patented by William Hyde Wollaston in 1806.

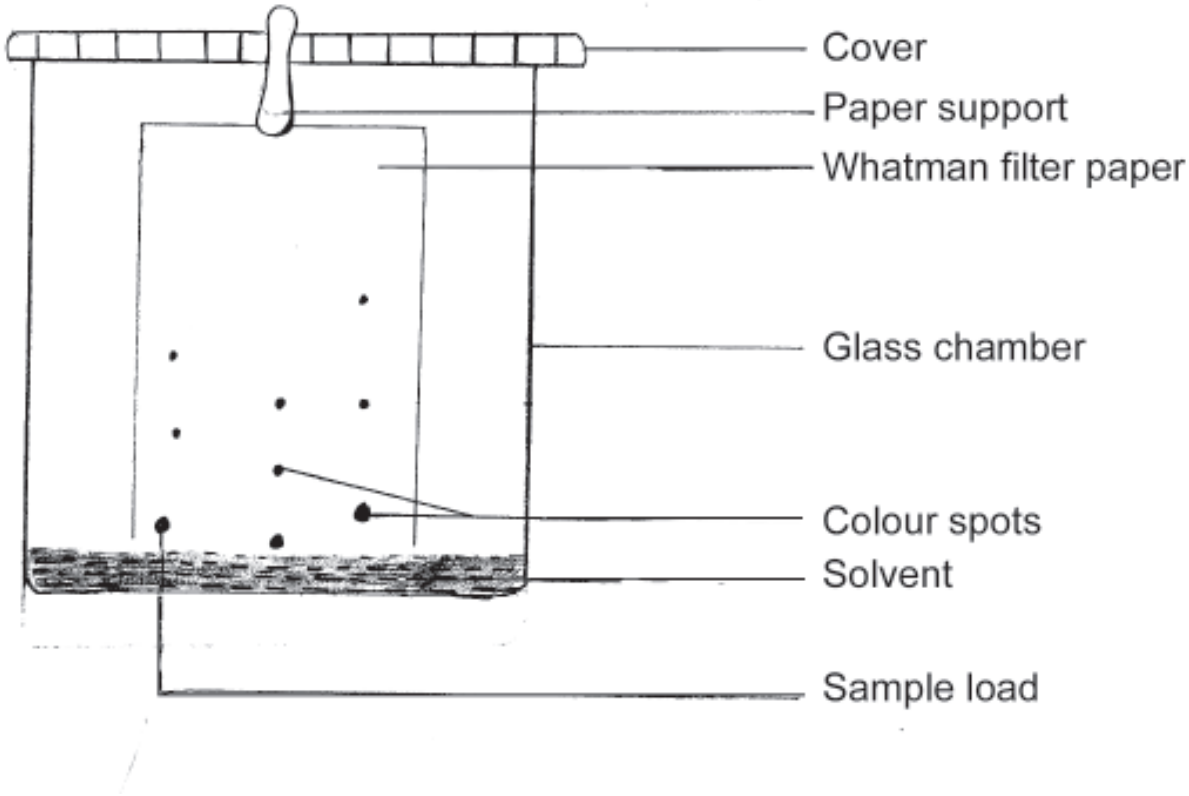
Mirror – type Camera lucida

Mirror- type camera lucida has three components, viz., an adjustable ring, a prism head and a reflecting mirror. The light rays passing through the ocular lens are deflected at 90º by the prism. The rays are further deflected at 90º by the mirror. This arrangement projects a virtual image on the desk-top beside the microscope. If a piece of paper is kept there, the observer can see the superimposed magnified image of the object on the paper through the ocular lens. The outline of the image can be done by free hand drawing.

BIOPHYSICS

 ISBN 978-81-958369-0-1

PAPER CHROMATOGRAPHY



PAPER CHROMATOGRAPHY

Paper chromatography is a special type of liquid- liquid partition chromatography. In this, stationary phase is a film of immobilized water, adsorbed on a special type of filter paper made of cellulose. The mobile phase is an organic solvent percolating over the stationary phase.

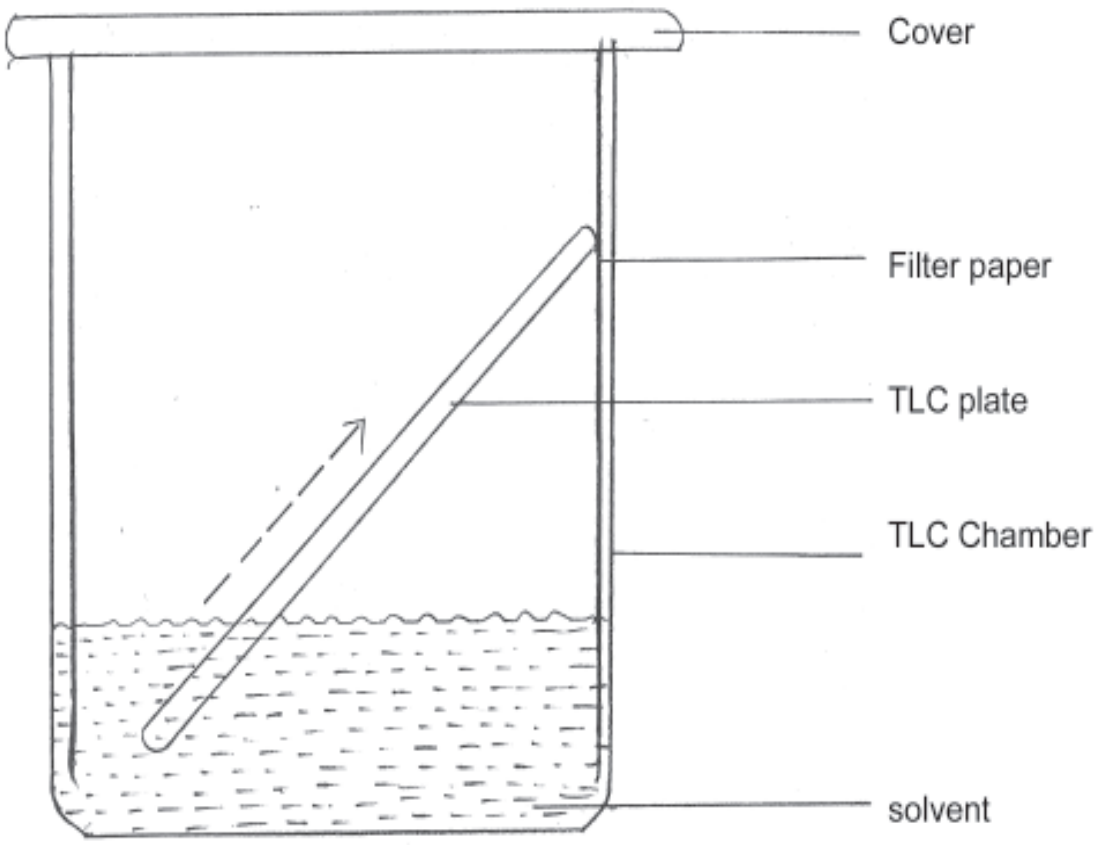
Working

The apparatus for paper chromatography consists of a support for holding the chromatographic paper, a solvent trough and an air – tight glass chamber. The sample to be separated is loaded near the lower edge of filter paper and dried. It is suspended in the glass chamber with the lower end just dipping in the solvent for running. The solvent ascends through the paper by capillary action, carrying along with it the various components of the sample. The components move at different rates based on their differential distribution between the two phases. When the solvent reaches three- fourth of the chromatogram, it is removed from chamber and dried. Colored spots can be visualised while colourless components are located by spraying a colouring reagent to form coloured complexes for visualization. The Resolution Factor of the separated components are calculated using the formula:

$$Rf = \frac{\text{Distance moved by solute (Solute front)}}{\text{Distance moved by solvent (Solvent front)}}$$

 ISBN 978-81-958369-0-1

THIN LAYER CHROMATOGRAPHY



THIN LAYER CHROMATOGRAPHY

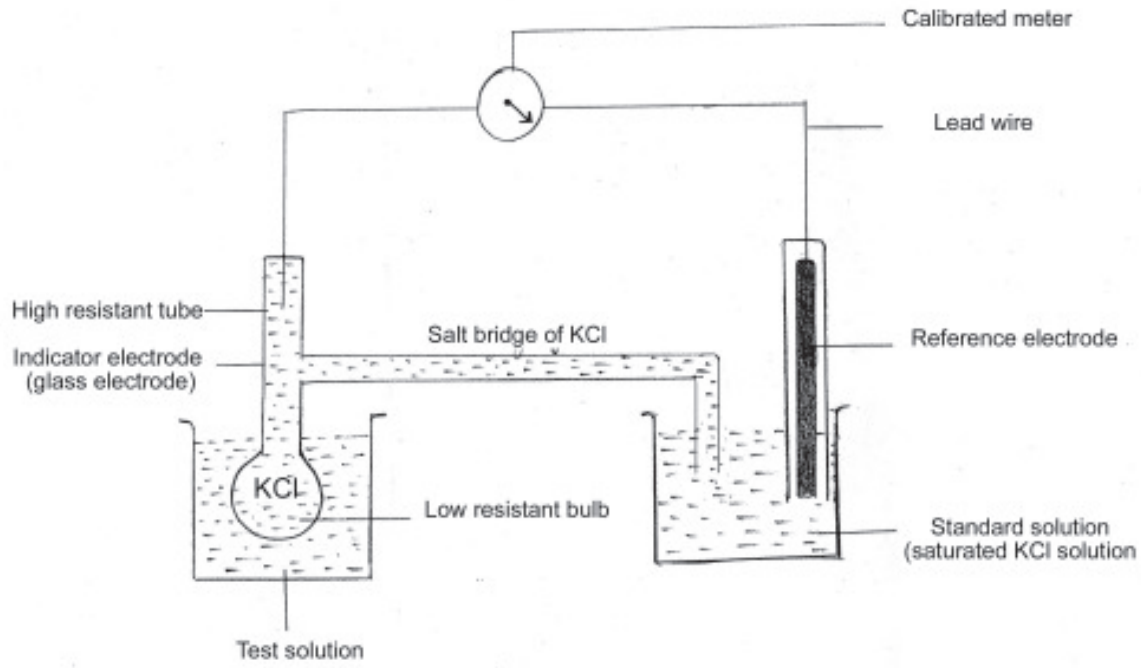
Thin-layer chromatography is a type of liquid – liquid partition chromatography. In this, the stationary phase is a film of immobilized water, adsorbed on a uniformly thin adsorbent layer of silica gel, alumina, cellulose etc. The adsorbent is coated on a glass plate that forms the supporting medium. The mobile phase is a suitable solvent, which percolates over the stationary phase. The differential distribution or partition of the sample components between the two immiscible phases bring about their differential migration and separation.

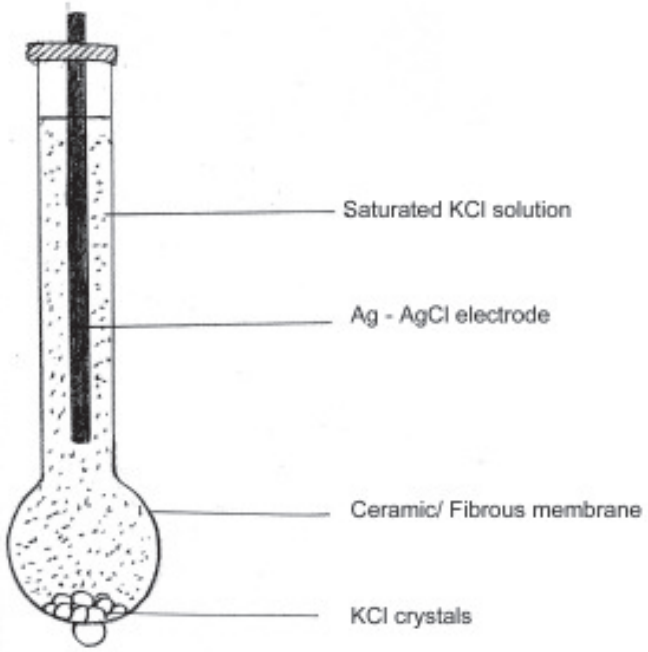
Working

The slurry of the adsorbent is spread evenly on the TLC plate. The sample is loaded near the lower end of the chromoplate and dried. The plate is placed in a slanting position in the solvent taken in TLC chamber such that the sample spot is above the level of the solvent. The tank is covered with a lid and made air-tight. The solvent ascends by capillary action carrying with it the components of the sample solution. The components move at different rates based on their differential distribution between the two phases. When the solvent reaches three- fourth of the chromatogram, it is removed from chamber and dried. Coloured spots can be visualised while colourless components are located by spraying a colouring reagent to form coloured complexes for visualization. The Resolution Factor of the separated components are calculated using the formula:

$$Rf = \frac{\text{Distance moved by solute (Solute front)}}{\text{Distance moved by solvent (Solvent front)}}$$

pH METER


Calibrated meter
Lead wire
High resistant tube
Indicator electrode
(glass electrode)
Salt bridge of KCl
Reference electrode
KCl
Low resistant bulb
Standard solution
(saturated KCl solution
Test solution


Saturated KCl solution
Ag - AgCl electrode
Ceramic/ Fibrous membrane
KCl crystals

pH METER

A pH meter is an electronic device for measuring the pH value of a solution. It has two main components-

1. A test cell or concentration cell and
2. A potentiometer

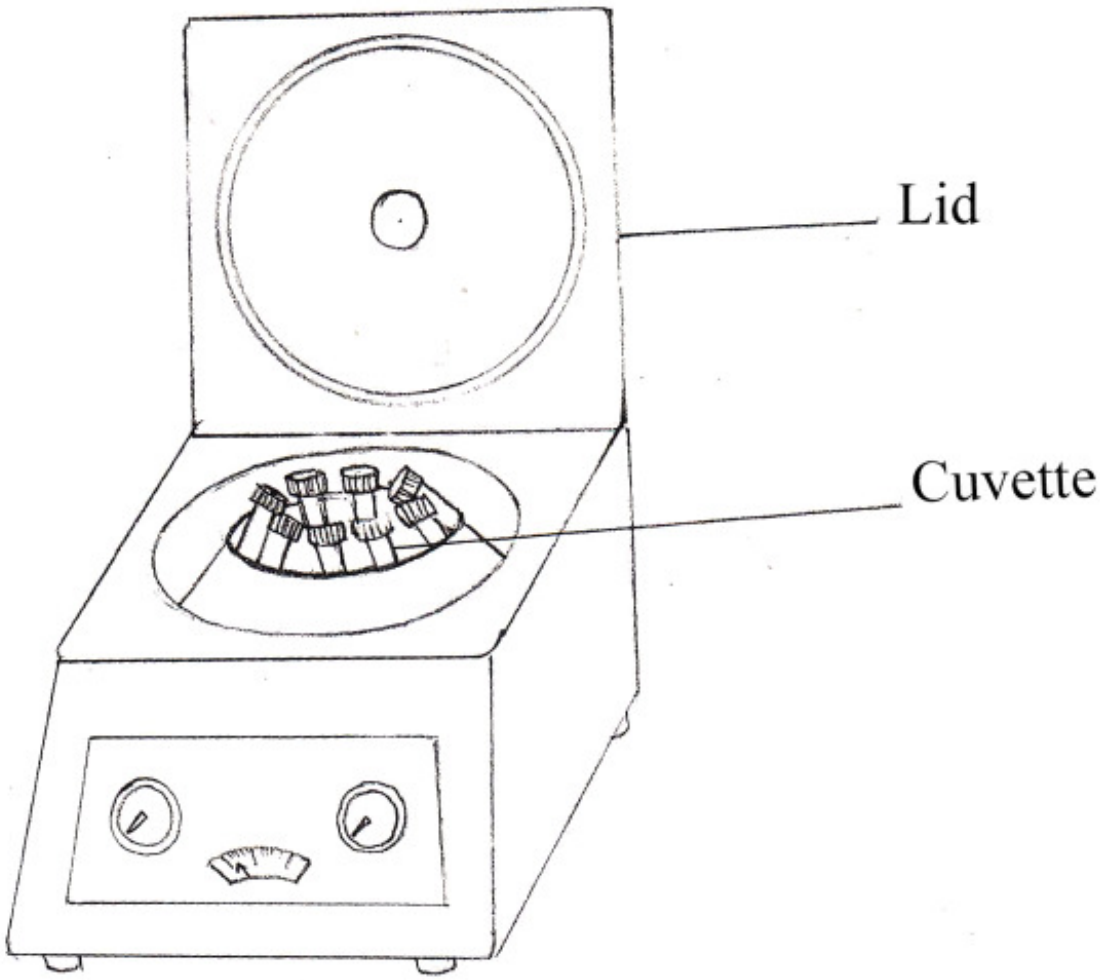
The test cell consists of a reference electrode and an indicator electrode. Ag- AgCl electrode is used as the most standard reference electrode. Glass electrode is commonly used indicator electrode. It consists of a high – resistance glass tube with a low – resistance bulb at it's bottom. The glass is permeable to H+ ions. Only the bulb is pH sensitive.

Working

When the glass electrode is dipped in the test solution, H+ ions from the standard solution (saturated solution of KCl that serves as salt bridge between electrodes) enter it through the glass bulb. The potential difference developed between the two electrodes is measured using calibrated potentiometer. This is proportionate to the pH value of the test solution.

 ISBN 978-81-958369-0-1

CENTRIFUGE



CENTRIFUGE

Centrifugation is the separation and sedimentation of sub-cellular fractions of the homogenate according to their size, mass, density and specific gravity. Centrifuge is the instrument used for centrifugation.

Working

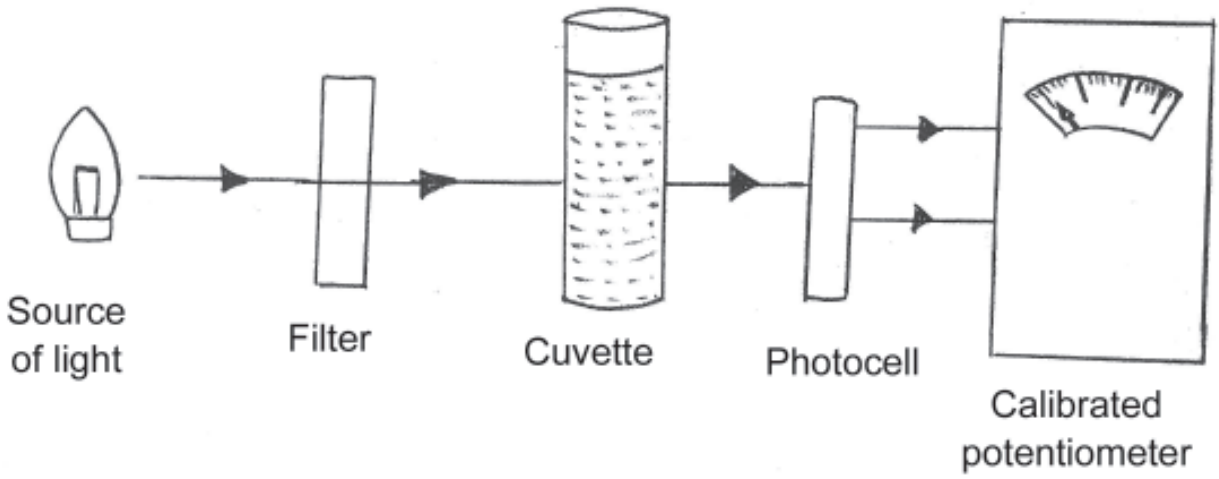
It consists of a pivoted cylinder called rotor in which samples are placed. The rotor rotates around a central axis at high speed that produces gravitational fields. The high rotational speed produces a centrifugal force which is directly proportional to the speed of the rotor and the radius of rotation. Relative Centrifugal Force (RCF) is the force acting on the samples during centrifugation. It is directly proportional to the number of revolutions per minute (ù), radius of rotation (r) and the gravitational force (g= 980 cm/ sec).

$$RCF = ù^2r/ g$$

A low- speed centrifuge is used for routine sedimentation of heavy particles. Their rotor has a maximum speed up to 5,000 rpm with RCF values upto 3000g. It is used at room temperature and have no temperature control device.

 ISBN 978-81-958369-0-1

PHOTOELECTRIC COLORIMETER



COLORIMETER

Colorimeter is an instrument used for measuring colour intensities of a solution by measuring the rate of absorbance of specific wavelengths of light by the solution. It gives accurate values about the optical density or percentage transmission of coloured solutions. It determines the concentrations of solutes in a solution by applying the Beer – Lambert's Law. The Law states that the intensity of a beam of monochromatic light passing through a solution decreases inversely with solute concentration, and decreases exponentially with an increase in the thickness of the solution.

PHOTOELECTRIC COLORIMETER

In Photoelectric colorimetry, the intensities of the colours of the standard and the test solutions are measured by keeping the wavelength and intensity of the incident light and thickness of both the solutions constant, and the distance traversed by light through the two solutions equal. The basic components of a photoelectric colorimeter are:

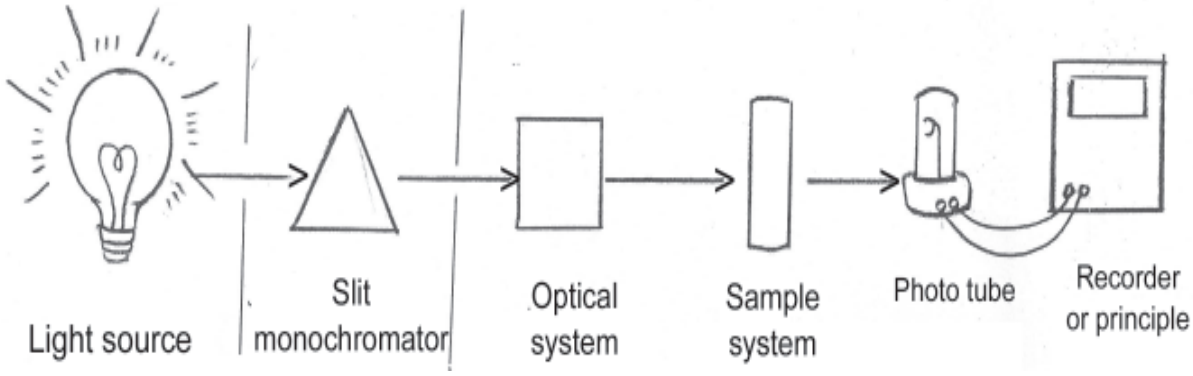
i. A light source
ii. Coloured Optic filters
iii. Cuvettes
iv. Photocell or phototube
v. Potentiometer

Working

Light rays from the electric lamp (tungsten filament) pass through a prism system to produce a spectrum. From this spectrum, the filters allow only specific wavelength to pass across them. The selected wavelength is transmitted to the test solution in the cuvette. The light emerging from the test solution is focused by a lens system to the photoelectric cell that converts light energy to electric energy. The potential difference generated by the photoelectric cell is measured by the potentiometer.

 ISBN 978-81-958369-0-1

SPECTROPHOTOMETER



SPECTROPHOTOMETER

Spectrophotometry is the technique used for the characterisation, identification and quantitative estimation of biomolecules in solution by measuring the light energy absorbed by the solution. It is used for the determination of the concentration of solute in a solution. It is based on Beer – Lambert's Law. The Law states that the intensity of a beam of monochromatic light passing through a solution decreases inversely with solute concentration, and decreases exponentially with an increase in the thickness of the solution. The major components are:

i. A light source .
   (Deuterium lamp for UV range, Tungsten- Halogen lamp for Visible range)
ii. A monochromator.
iii. An optical system of a series of slits, filters, mirrors and lenses.
iv. A sample chamber with cuvette.
v. A detector or phototube.
vi. A galvanometer or recorder.

Working

Light rays from the electric lamp pass through a prism system to produce a spectrum. From this spectrum, the monochromator allow only specific wavelength to pass across them. The selected wavelength is transmitted to the test solution in the cuvette. The light emerging from the test solution is focused by a lens system to the photoelectric cell that converts light energy to electric energy. The potential difference generated by the photoelectric cell is measured by the galvanometer.

LABORATORY MANUAL

FIRST DEGREE PROGRAMME (CBCSS) IN BOTANY UNIVERSITY OF KERALA

VOLUME I

This book entitled Laboratory Manual First Degree Programme in Botany (CBCSS) University of Kerala Volume I is prepared as a user-friendly reference material for the practical work of Under Graduate students in Botany as per the Syllabus of the University of Kerala. The book covers the topics in the first Semester and second Semester of First Degree Programme in Botany under Choice based Credit and Semester System. We tried to incorporate as much details as possible in the illustrations made in the Manual. The notes to each illustration needed for the practical examination are included. Hope this Laboratory Manual would be of great help to the students and teachers in their practicals.

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