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G.O No. 219 New Syllabus

BOTANY

UNIT-II

PTERIDOPHYTES, GYMNOSPERMS & PALEOBOTANY

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PTERIDOPHYTES

PG TRB (2025-2026)

UNIT- 2

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UNIT-2

PTERIDOPHYTES

SYLLABUS

PTERIDOPHYTES:

Classification of Pteridophytes (Reimer, 1954). Stelar evolution. Telome theory. Sorus – Origin, types and sporangial development. Heterospory and seed habit – Alternation of generations. Apogamy, Apospory and parthenogenesis in Pteridophytes. Comparative morphology, anatomy, reproduction and evolutionary studies of the following groups: Psilopsida, Lycopsida, Sphenopsida and Pteropsida. Economic importance of Pteridophytes.

GYMNOSPERMS:

General character, classification of gymnosperms (sporne, 1974). Origin and Evolution of gymnosperms. Comparative study of vegetative, anatomical and reproductive characteristics of Cycadales, Ginkgoales, Coniderales, Gnetales. Economic importance of gymnosperms.

PALEOBOTANY:

Concept of Paleobotany: Geological time scale. Contributions of Birbal Sahni, Technique for paleobotanical studies. Fossilization process, Types of fossils, the fossil records: systematic reconstruction and nomenclature of fossil plants, Determination of Age of Fossils, Fossil Pteridophytes: Lepidocarpon, Botrypoteris. Fossil Gymnosperms: Williamsonia and Cordaites.

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2. Razdan, M. K - Introduction to Plant tissue culture.

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6. Brown T.A - Genomes.

7.Satyanarayana. U - Biotechnology,

8. Neal Stewart. C - Plant Biotechnology And Genetics

9.T.A. BROWN - Gene Cloning And DNA Analysis

MORE REFERENCE

- PGTRB Previous Year Question Papers
- UGC NET Previous Year Question Papers

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UNIT – 2

1. PTERIDOPHYTES

1.1 INTRODUCTION

- The word Pteridophyta is of Greek origin. Pteron means "feather" and Phyton means plant. The plants of this group have feather like fronds (leaves). The group pteridophyta included in Cryptogams with Thallophyta (algae & fungi) and Bryophytes.
- The algae, fungi and bryophytes are called lower cryptogames (non- vascular cryptogams) while the Pteidophytesare called higher cryptogams (vascular cryptogames), because only pteridophytes have well developed conducting system among cryptogams. Due to this reason they are the first true land plants.
- All cryptogams reproduce by means of spores and do not produce seeds. The Peridophytes are assemblage of flowerless, seedless, spore bearing vascular plants that have successfully invaded the land.
- Pteridophytes have a long fossil history on our planet. They are known from as far back as 380 million years. Fossils of pteridophytes have been obtained from rock strata belonging to Silurian and Devonian periods of the Palaeozoic era.
- **(PG TRB 2015)** So the Palaeozoic era sometimes also called the "The age of pteridophyta". The fossil Pteridophytes were herbaceous as well as arborescent. The tree ferns, giant horse tails and arborescent lycopods dominated the swampy landscapes of the ancient age.
- The present day lycopods are the mere relicts the Lepidodendron like fossil arborescent lycopods. Only presentday ferns have nearby stature of their ancestors. Psilotum and Tmesipteris are two surviving remains of psilopsids, conserve the primitive features of the first land plants.

- In the plant kingdom, pteridophytes occupy a position in between bryophytes and gymnosperms, and therefore they have some similarities with the bryophytes on the one hand and with the gymnosperms on the other hand.

The similarities with bryophytes are

(i) presence of sterile jacket around the antheridium and archegonium,

(ii) requirement of water and moisture for the fertilization,

(iii) presence of alternation of generations,

(iv) formation of spores etc. while with gymnosperms

(i) sporophytic plant body and it's independent nature,

(ii) differentiation of sporophyte into root, shoot and leaves,

(iii) presence of vascular tissues for conduction etc.

- The presence of vascular elements in pteridophytes makes their grouping with gymnosperms and Angiosperms as Trachaeophyta. The reproduction by spores and similar events of life cycle place them among lower plants.
- The lower plants algae, fungi, bryophytes and pteridophytes were earlier grouped together as cryptogams. Bryophytes, Pteridophytes and Gymnosperms are also classified as Archegoniatae due to the presence of a common reproductive body archegonium.

1.1.1 GENERAL CHARACTERS OF PTERIDOPHYTES:

1. The main independent plant body is sporophyte with vascular system.

2. The pteridophytes grow mostly in cool, moist and shady places, but some are aquatic (Marsilea, Salvinia, Azolla etc.) and few are xerophytic (Selaginella rupestris, S. respanda, Marselia rajasthanensis, Marselia condenseta etc.).

3. Plants are differentiated into true roots, shoots and leaves. Some primitive members lack true roots and welldeveloped leaves (e.g.; in members of order Psilophytales and Psilotales).

4. Except few woody tree ferns all living pteritophytes are herbaceous.

5. They may be dorsiventral or radial in symmetry with branched stems.

6. The leaves of ptridophyte may be Scale like leaf (e.g. Equisetum), small sessile leaves (e.g., Lycopodium and Selaginella) and large, petiolate compound leaves occurs in true ferns.

7. The stem bears leaves which may be microphyllous type in which the leaves are quite small with unbranched midrib (e.g. Lycopodium, Selaginella, Equisetum), or megaphyllous type, in which the leaves are large with branched midrib (e.g. ferns).

8. In fern, the young leaves show circinate vernation (curved inwards).

9. Primary embryonic roots are short lived and replaced by adventitious roots.

10. The pteridophytes reproduce by haploid spores which are produced within a specialized structure called sporangium.

11. Plants may be homosporous (all spores are same in shape and size) and heterosporous (spores are of two different shape and sizes, smaller one called microspore and larger one megaspore).

12. In some pteridophytes the sporangia developed on stems in the axil between leaf and stem, or on leaves (mostly ventral surface of leaves). On the stem sporangia may be terminal e.g. Rhynia, lateral in Lycopodium, on the surface of leaves in Ferns. The sporangia borne on ventral side of specialized leaf and such leaf is called Sporophyll. In aquatic ferns micro and megasporangia together are covered by a common membrane and this bean shaped structure is called sporocarp.

13. In true ferns the sporangia are located on the lower surface of the leaf as clusters called sori (sorus).

14. The haploid spore is a unit of gametophyte. On germination it develops into gametophytic prothallus.

15. The Gametophytic plant is called prothallus since it more or less looks like the thallus of a primitive bryophytes.

16. Gametophyte bears sex organs archegonia and antheridia. As a result of fertilization the zygote or oospore is formed.

17. The homosporous plants are monoecious (antheridia and archegonia borne on same thallus).

18. Heterosporous plants are mostly dioecious (antheridia and archegonia borne on separate thalli).

19. Microspore gives rise to male prothallus which bears the male sex organs antheridia.

20. Megaspore gives rise to female prothallus which bears the female sex organs archegonia.

21. The sex organs are embedded or projected in the prothallus.

22. The male gametes are called antherozoids and produced inside the antherdium.

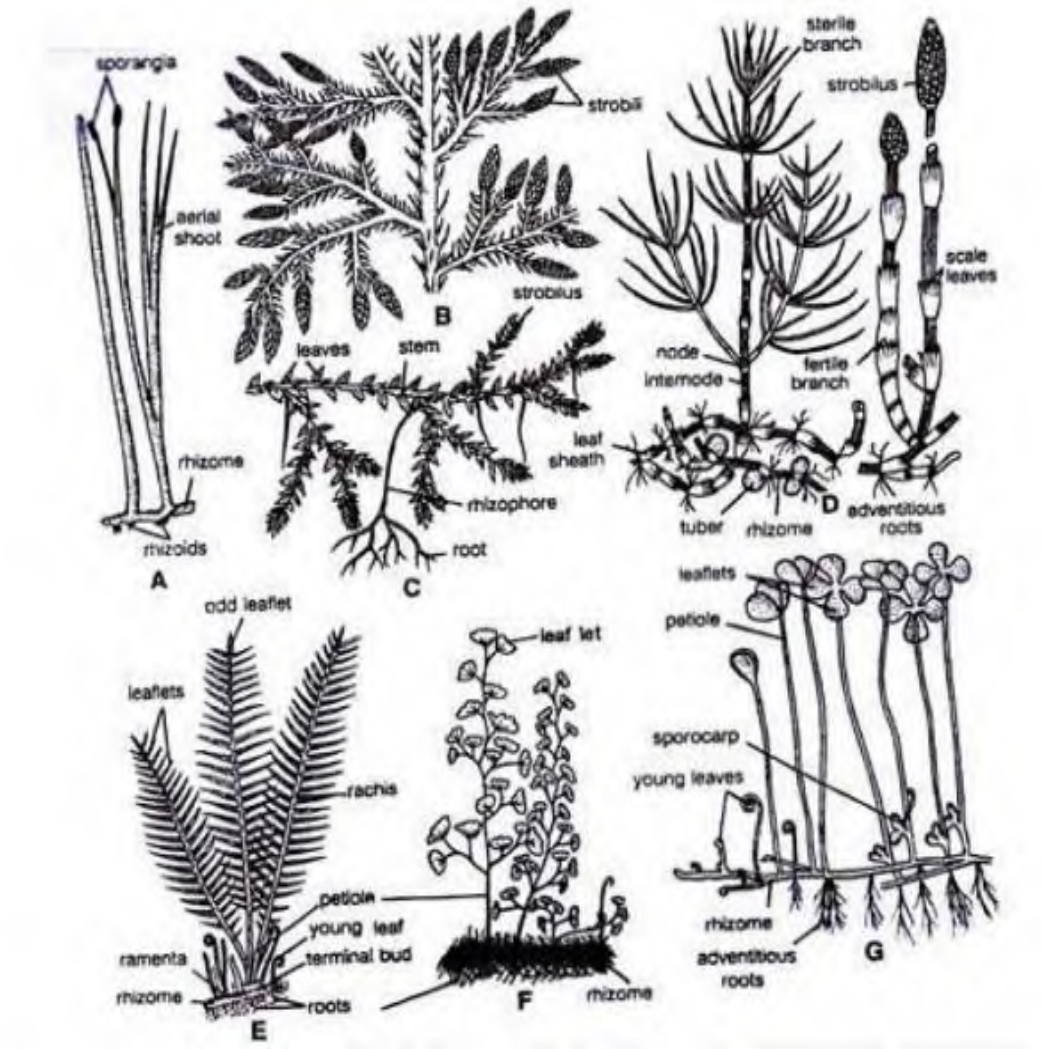
23. Antherozoids are unicellular, spirally coiled and flagellate.

24. The archegonia are flask shaped and differentiated into upper neck and lower broader venter.

25. The achegonial neck is projected and the venter is embedded.in the prothallus. 26. Water (moisture) is essential for completion of fertilization.

27. The egg and antherozoids fuse to form diploid zygote. The Zygote develops into new sporophytic plant body.

28. Clear alternation of generation takes place in the life cycle of Pteridophytes which is always heteromorphic type.



Here are some multiple-choice questions (MCQs) related to the characterization of pteridophytes:

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MULTIPLE CHOOSE QUESTIONS-1

1. Which of the following is a characteristic feature of pteridophytes?

A) Presence of seeds
B) Presence of flowers
C) Presence of vascular tissue
D) Lack of chlorophyll

2. Pteridophytes are classified under which of the following groups of plants?

A) Bryophytes
B) Gymnosperms
C) Angiosperms
D) Tracheophytes

3. What is the dominant generation in the life cycle of pteridophytes?

A) Gametophyte
B) Sporophyte
C) Zygote
D) Embryo

4. Which of the following is a common example of a pteridophyte?

A) Pine tree B) Fern C) Rose D) Grass

5. Pteridophytes have vascular tissue that includes:

A) Xylem and Phloem B) Xylem and Collenchyma

C) Xylem and Sclerenchyma D) Phloem and Parenchyma

6. What is the main function of the rhizoid in pteridophytes?

A) Reproduction B) Absorption of water and nutrients

C) Photosynthesis D) Transport of food

7. Pteridophytes reproduce by means of:

A) Seeds B) Spores C) Bulbs D) Flowers

8. Which of the following structures in pteridophytes produces spores?

A) Root B) Leaf

C) Strobilus or sporangium D) Flower

9. In pteridophytes, the male gamete is known as:

A) Oosphere B) Antheridium C) Archegonium D) Zygote

10. The vascular tissue in pteridophytes is arranged in:

A) Vascular bundles B) Continuous vessels

C) Simple tissue pattern D) None of the above

1.2 CLASSIFICATION OF PTERIDOPHYTES

- On the basis of presence and absence of seeds the vascular plants were classified by earlier taxonomists into two divisions, Pteridophyta and Spermatophyta. The division Pteridophyta included primitive vascular plants which bear no seeds.
- Later some fern like seed bearing fossil plants (Cycadofilicales) were discovered in 1903. The discovery eliminated the distinction between the two divisions Pteridophyta and Spematophyta.
- Sinnott (1935) therefore introduced a new term “Tracheophyta” for a division which includes all the vascular plants. Eames (1936) on the basis of some characters of plants and position of sporangia the division Tracheophyta divided into four groups, Psilopsida, Lycopsida, Sphenopsida and Pteropsida. Zimmermann (1930) and Arnold (1947) considered these groups as divisions and Tippo (1942) considered as subphyla.
- Riemer’s proposed a classification of Pteridophyta which was published in 1954 edition of Engler`s Syllabus der Pflanzen familien. Sporne (1966) also followed the same classification. Following is an outline of Riemer`s classification.

1.2.1 REIMERS CLASSIFICATION (1954)

- The **Reiner Classification** (1954) is a system that classifies pteridophytes based on **evolutionary relationships** and **morphological features**, primarily focusing on the structure of the plant and its reproductive organs.
- This classification was proposed by **Karl Reiner** in 1954. Here's an outline of the **Reiner classification (1954)** for pteridophytes:

Reiner's Classification (1954) of Pteridophytes

- Reiner's classification was a significant attempt to provide a natural and more systematic arrangement of the pteridophytes. It is based on **phylogenetic** and **morphological criteria**, with an emphasis on evolutionary development.
- The system classifies pteridophytes into **three main groups**:

1. Class Psilopsida (Order: Psilotales)

2. Class Lycopsida (Order: Lycopodiales)

3. Class Equisetopsida (Order: Equisetales)

4. Class Filicopsida (Order: Filicales)

1. Class Psilopsida (Order: Psilotales)

Characteristics:

- These are the most primitive group of pteridophytes.
- Lack true roots and leaves.
- Simple, dichotomously branching stems.
- Reproduce through spores.

Example Genera:

- Psilotum (commonly called whisk ferns).

Evolutionary Significance:

- Represents early vascular plants in evolutionary history.

2. Class Lycopsida (Order: Lycopodiales)

Characteristics:

- Possess microphylls (small, simple leaves).
- Presence of vascular tissue.
- Some members have strobili (cone-like structures) for spore production. Includes both extinct and extant species.

Example Genera:

- Lycopodium (club mosses).
- Selaginella (spike mosses).

Evolutionary Significance:

- The group shows more advanced features like vascular tissue and leaf structure, making it more evolutionarily advanced than Psilopsida.

3. Class Equisetopsida (Order: Equisetales)

Characteristics:

- Characterized by jointed stems and whorled leaves.
- Contains well-developed vascular tissue.
- Sporangia are borne in strobili at the tip of stems.

Example Genera:

- Equisetum (horsetails).

Evolutionary Significance:

- More specialized than Lycopsida, with distinctive features like jointed stems and specialized reproductive structures.

4. Class Filicopsida (Order: Filicales)

Characteristics:

- Known as ferns, these plants have large, compound leaves called **fronds**.

Fronds typically have sori (clusters of sporangia) on the underside.

Complex vascular tissue, with xylem and phloem arranged in bundles.

Example Genera:

- Pteridium (bracken ferns),
- Adiantum (maidenhair ferns).

Evolutionary Significance:

- The most advanced group of pteridophytes in terms of morphology, with well-differentiated leaves and complex reproductive mechanisms.

Key Points of Reiner's Classification:

- It focused on **evolutionary relationships** between the different groups of pteridophytes.
- It **highlighted the primitive nature** of Psilopsida and the more advanced structures seen in ferns and horsetails.
- The classification uses **morphological features** (such as leaf type, stem structure, and reproductive organs) as key differentiators.

Summary of Reiner's Classification of Pteridophytes

Class	Example	Key Features
Psilopsida	Psilotum	Lack of true roots and leaves, dichotomous branching, simple structure.
Lycopsida	Lycopodium, Selaginella	Microphylls, strobili (cone-like structures), vascular tissue, some extinct.

Equisetopsida	Equisetum (Horsetail)	Jointed stems, whorled leaves, strobili for spore production.
Filicopsida	Pteridium, Adiantum	Large fronds, sori on fronds, complex vascular system, most advanced group.

- This classification reflects a **phylogenetic perspective**, grouping pteridophytes based on their evolutionary relationships and structural complexity.
- Although more modern classifications (such as that by **Smith et al.** in the 21st century) have refined the pteridophyte phylogeny, Reiner's work was influential in establishing a **broad evolutionary framework** for these plants.

Division = PTERIDOPHYTES

A.	Sub – division	:	**Psilophytopsida**
	Order	:	Psilophytales
	Families	:	Rhyniaceae, Zosterophyllaceae, Psilophytaceae, Asteroxylaceae
B.	Sub – division	:	**Psilotopsida**
	Order	:	Psilotales
	Families	:	Psilotaceae, Tmesipteridaceae
C.	Sub – division	:	**Lycopsida**
	Order .1	:	Protolepidodendrales
	Families	:	Drepanophyceae, Protolepidodendraceae
	Order . 2	:	Lycopodiales
	Family	:	Lycopodiaceae
	Order. 3	:	Lepidodendrales
	Families	:	Lepidodendraceae, Bothrodendraceae, Sigillariaceae, Pleuromeiaceae
	Order. 4	:	Isoetales
	Family	:	Isoetaceae
	Order. 5	:	Selaginellales
	Family	:	Selaginellaceae
D.	Sub – division	:	Sphenopsida
	Order. 1	:	Hyeniales
	Families	:	Protohyeniaceae, Hyeniaceae
	Order. 2	:	Sphenophyllales
	Families	:	Sphenophyllaceae, Cheirostrobaceae
	Order. 3	:	Calamitales
	Families	:	Asterocalamitaceae, Calamitaceae

	Order. 4	:	Equisetales
	Family	:	Equisetaceae
E.	Sub – division	:	**Pteropsida**
a.	Class	:	**PRIMOFILICES**
	Order . 1	:	Cladoxylales
	Families	:	Cladoxylaceae, Pseudosporochnaceae
	Order . 2	:	Coenopteridales
	Families	:	Zygopteridaceae, Stauropteridaceae, Botryopteridaceae
b.	Class	:	**EUSPORANGIATE**
	Order. 1	:	Marattiales
	Families	:	Asterotheaceae, Angiopteridaceae. Marattiaceae, Danaeaceae, Christenseniaceae
	Order. 2	:	Ophioglossales
	Family	:	Ophioglossaceae
c.	Class	:	OSMUNDIDAE
	Order	:	Osmundales
	Family	:	Osmundaceae
d.	Class	:	**LEPTOSPORANGIATE**
	Order . 1	:	Filicales
	Families	:	Schizaceae, Gleicheniaceae, Hymenophyllaceae, Dicksoniaceae, Matoniaceae, Dipteridaceae, Cyatheaceae, Dennstaedtiaceae, Adiantaceae, Polypodiaceae
	Order. 2	:	Marsileales
	Families	:	Pilulariaceae, Marsileaceae
	Order . 3	:	Salviniales
	Families	:	Salviniaceae, Azollaceae.

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MULTIPLE CHOOSE QUESTIONS-2

1. Pteridophytes are classified into how many major classes?

A) Two B) Three C) Four D) Five

2. Which of the following is NOT one of the major classes of pteridophytes?

A) Psilopsida B) Lycopsida C) Sphenopsida D) Gymnospermae

3. The class Psilopsida is characterized by:

A) Large, branched stems with well-developed roots and leaves

B) Small, leafless, and dichotomously branching stems

C) Presence of true leaves, roots, and cones

D) Presence of large fronds and spores

4. The plants belonging to the class Lycopsida are commonly known as:

A) Ferns B) Horsetails C) Club mosses D) Quillworts

5. Which of the following is a characteristic feature of plants in the class Sphenopsida?

A) Presence of sporangia on cones
B) Hollow, jointed stems with whorls of leaves
C) Dichotomous branching of the stem
D) Presence of fronds and sori

6. The plants of class Lycopsida mainly reproduce by:

A) Spores produced in strobili
B) Sexual reproduction through gametes
C) Asexual reproduction through rhizomes
D) Spores produced in sori

7. In which class of pteridophytes is the sporophyte generally the dominant form, characterized by well-differentiated roots, stems, and leaves?

A) Psilopsida B) Lycopsida C) Sphenopsida D) Pteropsida

8. Which of the following is the main characteristic of plants belonging to Psilopsida?

A) Vascular tissue arranged in a ring B) Branching stems and large leaves
C) Lack of leaves and roots D) Presence of large, compound leaves

9. Which class of pteridophytes includes ferns and their relatives?

A) Psilopsida B) Lycopsida C) Sphenopsida D) Pteropsida

10. The plants in Lycopsida are typically characterized by:

A) Large leaves arranged in a spiral
B) Presence of vascular bundles scattered in the stem
C) Simple, small leaves known as microphylls
D) Leaflets that are compound in nature

1.3 STELAR EVOLUTION THE STELE

- The term stele (Greek word meaning pillar) was used for the first time by Van-Tieghem and Douliot (1886) to designate the unit of vascular system. It is applicable only to the primary vascular of shoot and root, particularly of lower plants such as pteridophytes.
- However, in case of higher plants (such as gymnosperms and angiosperms) the term stele cannot be generally applied. Esau has suggested the term “primary vascular system” or the “vascular cylinder” in such cases.
- The stele may be defined as the central core of axis which includes primary vascular tissues (xylem and phloem) with or without pith and some surrounding fundamental tissues, such as pericycle.

- Some workers include endodermis as an outermost layer of stele whereas others consider it as the innermost layer of cortex and not included in the stele.

1.3.1 Types of stele and evolution of stelar system.

- The simplest and most primitive type of stele consists of a central solid core of xylem surrounded by phloem followed by pericycle. Such stele is called protostele.
- The other types of steles (such as siphonostele, dictyostele, etc.,) have been derived from it by appearance of pith or leaf and branch gaps during the course of evolution. Jeffrey recognised two main types of steles in pteridophytes
- (i) Protostele and (ii) Siphonostele

(1) Protostele:

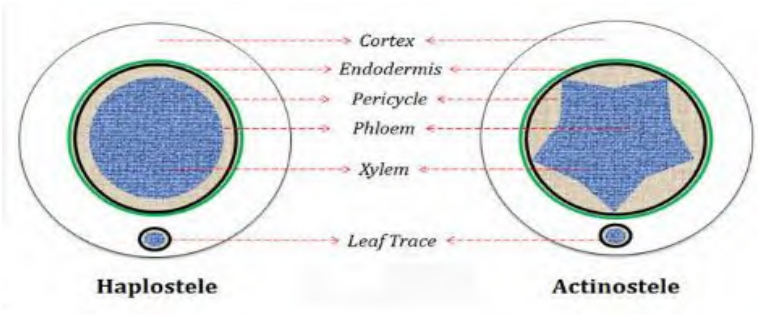
- It is the simplest and most primitive type of stele found in pteridophytes. It consists of central solid core of xylem surrounded by pholem followed by pericycle. Such a stele is found in the juvenile stage of sporophytes in almost all the pteridophytes. Eg., Lycopodium, Selaginella, psilotum etc. Breliner (1902) has divided the protostele into two types
- (i) Haplostele and (ii) Actinostele.

(i) Haplostele:

- It is the simplest type of protostele in which the central solid core of xylem is surrounded by a uniform layer of pholem. The pith is absent. The protoxylem usually faces towards periphery. The haplostele may be monarch, diarch or polyarch depending upon the number of protoxylem points.
- This type of stele is found in primitive fossil forms of psilophyta like Rhynia, Horneophyton and a number of living pteridophytes eg., Selaginella kraussiana, S. chrysocaulos, S. martensii, S. chrysorhiyos, etc. in few cases the protoxylem lies in the centre and is surrounded completely by metaxylem, ie., mesarch (eg., S. selaginoides in the creeping part of stem).

(ii) Actinostele:

- It is derived from haplostele. In this case the xylem is angular with radiating arms. The protoxylem lies at the tip of each arm. The xylem is surrounded by pholem. The phloem is not continuous, but occurs in patches in the concavities of star shaped xylem eg., Lycopodium serratum. Psilotum and the stem tips of Selaginella selaginoides.



- In a few cases the radiating arms of xylem deepen further so that the xylem becomes star – shaped in transverse section. The phloem invaginates deeper into the concavities. Such a stele is called stelate actinostele eg., Lycopodium annotinum.

(iii) Plectostele:

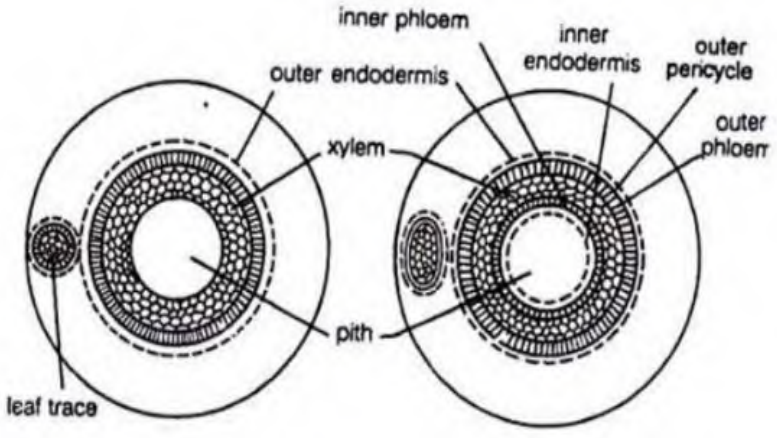
- In some cases the xylem becomes dissected into several plates like structures. The xylem, in a transverse section appears in the form of seperated parallel plates with phloem occupying the spaces between the two plates. Such a protostele is called plectostele, eg., Lycopodium clavatum and L. volubile.

(iv) Mixed protostele:

- In some cases the xylem becomes dissected into several pieces. In a transverse section it appears as if the xylem strands are embedded in the ground tissue of phloem. Such condition of stele is called mixed protostele. Eg., Lycopodium cernuum, Gleichenia dichotoma etc.,

(2) Siphonostele:

- A siphon (or pipe) like stele in which the centre is occupied by pith surrounded by xylem, phloem and pericycle, is called siphonostele. It is formed by medullation (or appearance of pith) in the protostele. The pith may be parenchymatous, sclerenchymatous or a hollow cavity.



- There are controversial views regarding the origin of pith in siphonostele. Two major views have been forwarded –

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UNIT V

CELL BIOLOGY AND MOLECULAR BIOLOGY

SYLLABUS

CELL BIOLOGY:

CELL STRUCTURE, ORGANIZATION OR PROKARYOTIC AND EUKARYOTIC CELLS. CELL THEORY, ULTRASTRUCTURE AND MOLECULAR ORGANIZATION OF CELL WALL, PLASMA MEMBRANE, CYTOPLASM, PROTOPLASM, ENDOPLASMIC RETICULUM, VACUOLES, NUCLEUS. CELL DIVISION AND CELL CYCLE: MITOSIS AND MEIOSIS THEIR REGULATION, STEPS IN CELL CYCLE AND CONTROL OF CELL CYCLE. ORGANISATION OF NUCLEAR GENOME: DNA AS GENETIC MATERIAL – PROKARYOTIC AND EUKARYOTIC DNA – CHROMATIN – CHROMOSOMES – GENE TRANSPOSON. REPLICATION OF DNA AND TYPES.

MOLECULAR BIOLOGY:

TRANSCRIPTION IN PROKARYOTES AND EUKARYOTES (RNA SYNTHESIS – ENZYMOLOGY – SIGNALING) – MECHANICS OF INITIATION, ELANGATION, TERMINATION – POST – TRANSCRIPTIONAL MODIFICATIONS AND RNA SPLICING – REGULATION OF GENE EXPRESSION (LAC AND TRP OPERONS) – RNA INTERFERENCE (TGS AND PTGS) – TRANSLATION (GENETIC CODE – REDUNDANCY AND ELUCIDATION OF BASE COMPOSITION – tRNA CHARGING – INITIATION, ELONGATION AND TERMINATION) – POST TRANSLATIONAL MODIFICATIONS. MOLECULAR CHAPERONES – HEAT SHOCK PROTEINS. BIOINFORMATICS: CONCEPTS, SCOPE AND APPLICATIONS.

REFERENCE BOOK:

1) De Robertis - Cell Biology,

2) Gerald Karp - Cell and Molecular Biology Concepts and Experiments

3) Wilson &Marrison - Cytology,.

4) Ajoy Paul - Text Book of Cell and Molecular Biology-Books

5) Sharp - Fundamentals of Cytology

6) P.S.Verma &V.K.Agarwal - Cell Biology, Genetics, Molecular Biology, Evolution and Ecology

7) T.A. Brown - Molecular Biology,

8) J.D. Watson, N.H. Hopkins, J.W. Roberts, J.A. Steltz & A.N. Weiner - Molecular Biology of gene.

9) R.W. Old & S.B. Primrose - Principles of Gene Manipulation

MORE REFERENCE:

- PG TRB Previous Year Question Paper
- UGC NET Previous Year Question Paper

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UNIT - V

1. CELL BIOLOGY

INTRODUCTION

- A **cell** is the smallest unit that exhibits all of the qualities associated with the living state. Cells must obtain energy from an external source to carry on such vital processes as growth, repair, and reproduction.
- All of the chemical and physical reactions that occur in a cell to support these functions constitute its **metabolism**. Metabolic reactions are catalyzed by **enzymes**. Enzymes are protein molecules that accelerate biochemical reactions without being permanently altered or consumed in the process. The structure of each enzyme (or any other protein) is encoded by a segment of a deoxyribonucleic acid (DNA) molecule referred to as a **gene**.
- **Molecular and cell biology** are the sciences that study all life processes within cells and at the molecular level. In doing so, these sciences draw upon knowledge from several scientific disciplines, including biochemistry, cytology, genetics, microbiology, embryology, and evolution.

1.1 CELL STRUCTURE

1.1.1 INTRODUCTION

- The cell is defined by Schultze (1861) as "a lump of protoplasm with a nucleus with it." However, this old definition is no more appropriate as we see in the cell a specific, well-ordered, living system, constituting in itself a harmonius organic unity, capable of storing energy and reproducing new ones.
- Therefore, Grundmann (1966) defined, "The cell is the smallest functional unit, composed of elements which counter-balance one another in an endogenous

equilibrium and complement one another to carry on the activities of life: metabolism, autochthonous reproduction and specific responses to stimuli".

- Perhaps, this can be the best definition. There are others, such as Lowey and Siekevitz (1963) who defined the cell as "a unit of biological activity delimited by a selective permeable membrane and capable of self Swanson (1972) the cell or protoplast may be defined as a piece of nucleated cytoplasm surrounded by a cell wall or membrane, existing singly or in groups and containing structures of various sorts."
- The above definitions though attempt to define cell as clearly as possible, are incomplete as it is difficult of define cell because it represents an abstract generalization that attempts to cover a field which is too complex.

1.1.2 CONCEPT OF MODERN CELL

1.SHAPE:

- When we examine different types of cells, an amazing variation is found in the shape which is due to the plastic nature of cytoplasm having no definite shape.
- The shape of the cells is dependent on the functional and structural adaptations and partly on the surface tension and viscosity of the cytoplasm, the influence of the mechanical action exerted by the neighbouring cells, and the rigidity of the cell membrane.
- Apart from these factors, the microtubules present within the cell also have an important influence on the shape.
- Some cells like Amoebae and white blood corpuscles have no definite shape and changer their appearance very frequently while other cells such as spermatozoa, erythrocytes, nerve cells and most plant cells possess a definite shape throughout their life span.
- In a liquid medium, due to the surface tension, most of the cells appear spherical. Majority of plant and animal cells are polyhedral due to reciprocal pressures. The shape of the cells varies from individual to individual and from one organ to other.
- Variation in shape is observed in different cells of even the same organ. In general, the shape is dependent on the functions performed by the cell.
- Muscle and nerve cells are elongated while epithelial cells are flattened. The shape of the cell may be irregular, triangular, polygonal, cuboidal, tubular, cylindrical, oval, rounded or elongated in various plants and animals gives the shape if some important types of cells.

2.SIZE OF CELL

A great variation is also evidenced in the size of the cells. Some cells like the eggs of bird's range upto a few centimeters but these are exceptions. Mostly cells are microscopic in size.

The smallest free-living organisms are Mycoplasmas

Shape of some representative types of cells of animals and plants.

Name	Shape	Examples
	Animals	
1. Epithelial	Cuboidal or brick shaped	Epidermid, glandular lining
2. Nervous	Cell body with elongated	Sensory and motore fibres
3. Mnuscular	Spindle	Smooth, striated muscles
4. Blood	Disk shaped or amoeboid in fluid	Erythrocytes and leucocytes
5. Connective	Spheroid cells	Cartilage, bone and connective tissue Cells
6. Sperm	Flagellated cells	Seaurchin sperm
7. Eggs	Spherical	Searchin and frog eggs
8. Protozoans	Diverse	Amoeba, Paramecium
	Plants	
1. Unicelluar algae	Diverse	Chlorella
2. Unicellular fungi	Diverse	Yeast
3. Bacteria	Diverse	Eisherichia coli
4. Filamentous fungi	Elongated cells	Neurospora
5. Epidermal	Cuboidal	Epidermis
6. Vascular	Elongate, spiral, disc-like	Phleom
7. Supporting	Elongated	Bast fibers
8. Parenchyma	Spheroidal	Mesophyll, interstitial cells etc.

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The following table gives an idea of the diversity in the size if the different types of cells.

Variation in size of different types of cells.

Cell organism	Size (Mass in grams)
1. Mycoplasma laidlawii (diameter 0.1 μ m)	10-16
2. Small bacteria	10-14
3. Pus and tubercle bacteria	10-12
4. Anthrax bacillus	10-11
5. Frog erythrocyte, malarial parasite, human Sperm, small protozoans	10-9
6. Entamoeba	10-8
7. Humal liver cell and smooth muscle fiber vorticella	10-7
8. Paramecium (60 cm diameter)	10-6
9. Human ovum	10-5
10. Frog eggs	10-2 to 10-3
11. Ostrich eggs, Cycas ovules (diameter 20 cm)	10-2 to 103

- The smallest cells are only 1,000 A0 (1 A0 = 10-6 microns or 10-6 meter) in diameter and are isolated from soil.
- There are responsible for a number of human infections. Iwanowsky (1892) first demonstarated these pleuropneumonia type organisms (PPLOs) passing through filters used for trapping bacteria.
- The bacteria range between 5,000 A0 for the cocci and 20 in length for the filamentous bacteria. The blue green algae occur and 20 in length for the filamentous bacteria. The unicellural organisms, diatoms are upto 100 or more in length. Amoeba is about 1 mm. (1,000) in length. Euglena is about 0.5mm.
- The cells of the tissues of higher plants and animals vary between 20-30. The human ovum is 200 in diameter, while that of an ostrich, perhaps the biggest single cell is about 5 cm. indiameter.

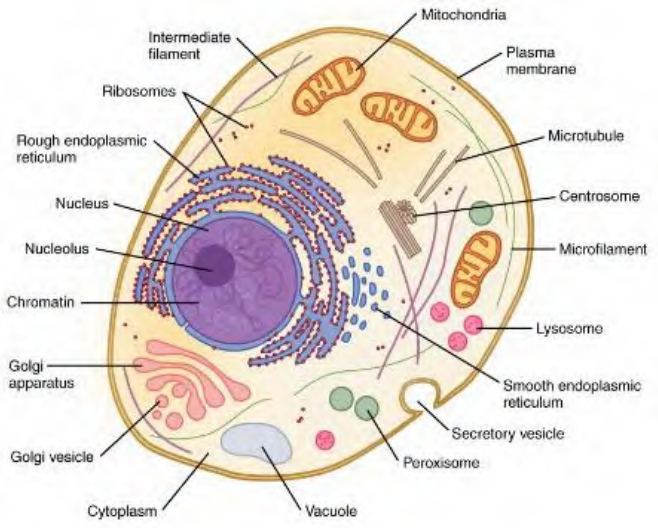
The size of the cell is influenced by the nucleus, as it regulates most of the activities in the cytoplasm, so that there is a limit to the amount of cytoplasm which a particular nucleus can control. This problem is solved to some extent by multinucleate forms such as the amoeba, Chaos chaos etc.

1.1.3 CELL STRUCTURE

The cell is the basic structural, functional, and biological unit of all known living organisms. A cell is the smallest unit of life. Cells are often called the "building blocks of life". The study of cells is called cell biology. Cells are the basic building blocks or structural and functional unit of all living beings. The human body is composed of trillions of cells.

They provide structure for the body, take in nutrients from food, convert those nutrients into energy, and carry out specialized functions. Core organelles are found in virtually all eukaryotic cells. They carry out essential functions that are necessary for the survival of cells – harvesting energy, making new proteins, getting rid of waste and so on. Core organelles include the nucleus, mitochondria, endoplasmic reticulum and several others.

1. Cell membrane:



The cell membrane (also known as the plasma membrane or cytoplasmic membrane, and historically referred to as the plasmalemma) is a biological membrane that separates the interior of all cells from the outside environment (the extracellular space). [Singleton P] It consists of a lipid bilayer with embedded proteins. The

basic function of the cell membrane is to protect the cell from its surroundings. The cell membrane controls the movement of substances in and out of cells and organelles. In this way, it is selectively permeable to ions and organic molecules.

It is composed of four different types of molecules:

- Phospholipids
- Cholesterol
- Proteins
- Carbohydrates.

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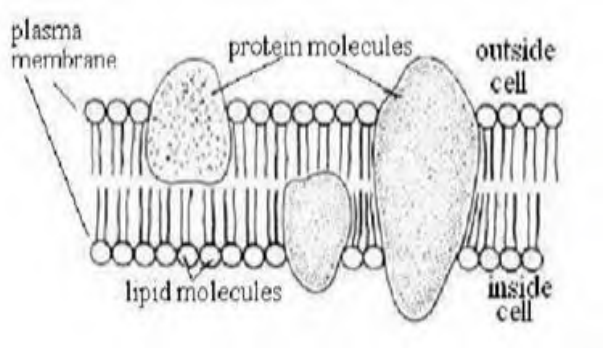
Functions:

The main functions of the cell membrane are:

- It is a physical barrier to maintain the physical integrity of the cell - that is to mechanically enclose the contents of the cell, and also
- It controls the movement of particles e.g. ions or molecules, into and out of the cell. Passive osmosis and diffusion: Some substances (small molecules, ions) such as carbon dioxide (CO2) and oxygen (O2), can move across the plasma membrane by diffusion
- It regulates exchange of materials with its surroundings.
- **Bulk Transport: Exocytosis** is the process by which a cell moves the contents of secretory vesicles out of the cell via the cell membrane.
- **Endocytosis** is the opposite process by which the contents of secretory vesicles are moved into the cell via the cell membrane.



2. Cytoplasm:

In cell biology, the cytoplasm is the material within a living cell, excluding the cell nucleus. It comprises cytosol (the gel-like substance enclosed within the cell membrane) and the organelles – the cell's internal sub-structures. All of the contents of the cells of prokaryotic organisms (such as bacteria, which lack a cell nucleus) are contained within the cytoplasm. Within the cells of eukaryotic organisms, the contents of the cell nucleus are separated from the cytoplasm, and are then called the nucleoplasm. The cytoplasm is about 80% water and usually colorless.

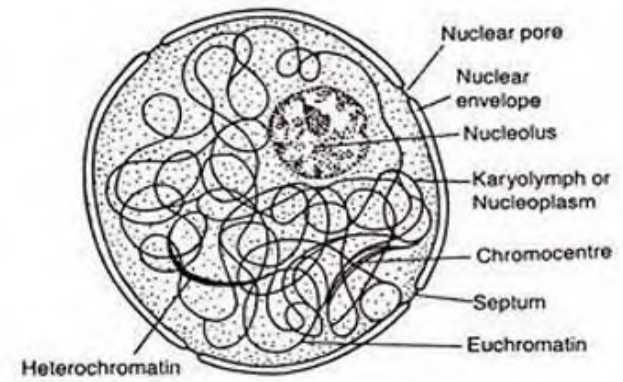
Function of Cytoplasm:

- Most of the important activities of the cell occur in the cytoplasm. Cytoplasm contains molecules such as enzymes which are responsible for breaking down waste and also aid in metabolic activity.
- Cytoplasm is responsible for giving a cell its shape. It helps to fill out the cell and keeps organelles in their place. Without cytoplasm, the cell would be deflated and materials would not be able to pass easily from one organelle to another.
- Cytosol is the part of the cytoplasm that does not contain organelles. Instead, cytosol is confined by the boundaries of a matrix which fills the part of the cell that does not contain organelles.

3. Nucleus:

The nucleus is an organelle found in eukaryotic cells. Inside its fully enclosed nuclear membrane, it contains the majority of the cell's genetic material. This material is organized as DNA molecules, along with a variety of proteins, to form chromosomes. The nucleus contains:

Nuclear membrane - The nuclear envelope, also known as the nuclear membrane, encloses the nucleus and nucleolus.



Structure of a nucleus.

Nucleoplasm - also known as karyoplasm is the matrix present inside the nucleus.

Chromatin Reticulum - Chromosomes are present in the form of strings of DNA and histones (protein molecules) called chromatin.

Nucleolus - The nucleolus (plural nucleoli) is a dense, spherical-shaped structure present inside the nucleus.

Function:

- The many pores in the nuclear membrane allow it to decide what enters and exits the nucleus.
- The nucleolus makes ribosomes. This is a very important job inside of the cell.

- Chromosomes contain the genetic information (DNA) of the cell. The chromosomes are the code for all of the functions that occur in a cell.
- Storage of hereditary material, the genes in the form of long and thin DNA (deoxyribonucleic acid) strands, referred to as chromatin.
- Storage of proteins and RNA (ribonucleic acid) in the nucleolus.
- Nucleus is a site for transcription in which messenger RNA (mRNA) are produced for protein synthesis.
- Exchange of hereditary molecules (DNA and RNA) between the nucleus and the rest of the cell.
- During the cell division, chromatins are arranged into chromosomes in the nucleus.
- Production of ribosomes (protein factories) in the nucleolus.
- Selective transportation of regulatory factors and energy molecules through nuclear pores.
- The main function of the cell nucleus is to control gene expression and mediate the replication of DNA during the cell cycle.

4. Mitochondrion:

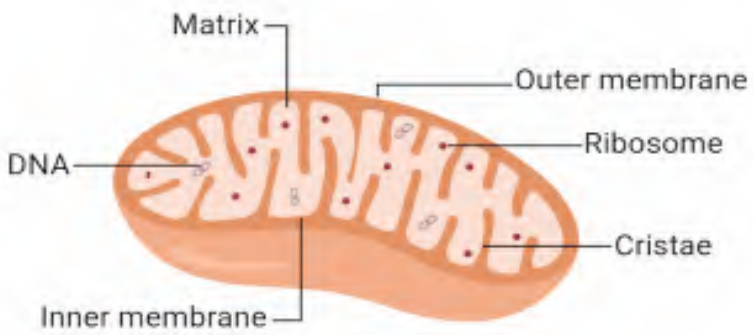
The mitochondrion is a double-membrane-bound organelle found in most eukaryotic organisms. Mitochondria generate most of the cell's supply of adenosine triphosphate (ATP), used as a source of chemical energy.

Structure:

The number of mitochondria in a cell can vary widely by organism, tissue, and cell type. For instance, red blood cells have no mitochondria, whereas liver cells can have more than 2000. The organelle is composed of compartments that carry out specialized functions. These compartments or regions include the outer membrane, the intermembrane space, the inner membrane, and the cristae and matrix. [Voet D.] A mitochondrion contains outer and inner membranes composed of phospholipid bilayers and proteins.

The two membranes have different properties. Because of this double-membraned organization, there are five distinct parts to a mitochondrion. They are:

- the outer mitochondrial membrane,
- the intermembrane space (the space between the outer and inner membranes),
- the inner mitochondrial membrane,
- the cristae space (formed by infoldings of the inner membrane), and
- the matrix (space within the inner membrane).
- Mitochondria stripped of their outer membrane are called mitoplasts.



Function

The most prominent roles of mitochondria are to produce the energy currency of the cell and to regulate cellular metabolism.

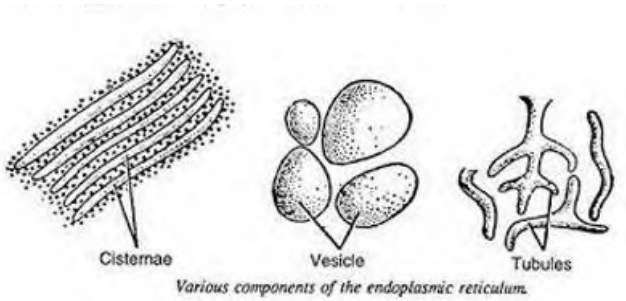
- Energy conversion
- Storage of calcium ions
- Regulation of the membrane potential
- Calcium signaling
- Regulation of cellular metabolism
- Steroid synthesis.
- Hormonal signaling

5. Endoplasmic reticulum (ER):

- The endoplasmic reticulum (ER) is a type of organelle found in eukaryotic cells that forms an interconnected network of flattened, membrane-enclosed sacs or tube-like structures known as cisternae. The membranes of the ER are continuous with the outer nuclear membrane. The general structure of the endoplasmic reticulum is a network of membranes called cisternae. These sac-like structures are held together by the cytoskeleton. The phospholipid membrane encloses the cisternal space (or lumen), which is continuous with the perinuclear space but separate from the cytosol.

Functions:

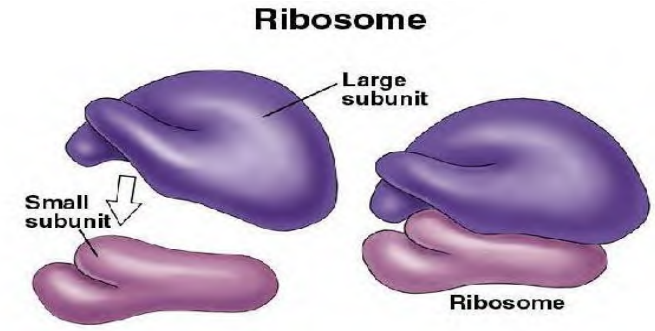
- The endoplasmic reticulum serves many general functions, including the folding of protein molecules in sacs called cisternae and the transport of synthesized proteins in vesicles to the Golgi apparatus. [Ozcan U.]
- Synthesize lipids, oils, Phospholipids.
- Synthesize of Steroids and Sex hormones.
- Hydrolysis of glycogen.



Various components of the endoplasmic reticulum.

6. Ribosome:

A ribosome is a complex cellular mechanism used to translate genetic code into chains of amino acids. Long chains of amino acids fold and function as proteins in cells. The ribosomes and associated molecules are also known as the translational apparatus. The ribosome is a highly complex cellular machine. It is largely made up of specialized RNA known as ribosomal RNA (rRNA) as well as dozens of distinct proteins (the exact number varies slightly between species). The ribosomal proteins and rRNAs are arranged into two distinct ribosomal pieces of different size, known generally as the large and small subunit of the ribosome.



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Function:

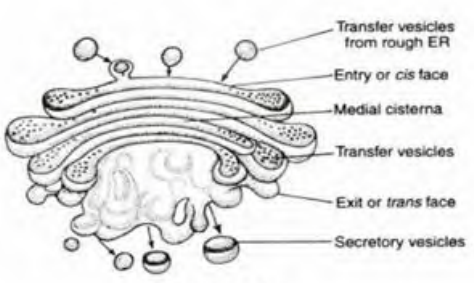
Ribosomes are minute particles consisting of RNA and associated proteins that function to synthesize proteins. Proteins are needed for many cellular functions such as repairing damage or directing chemical processes. Ribosomes can be found floating within the cytoplasm or attached to the endoplasmic reticulum.

7. Golgi apparatus:

The Golgi apparatus, also known as the Golgi complex, Golgi body, or simply the Golgi, is an organelle found in most eukaryotic cells. [Pavelk M. 2008]. In most eukaryotes, the Golgi apparatus is made up of a series of compartments and is a collection of fused, flattened membrane-enclosed disks known as cisternae (singular: cisterna, also called "dictyosomes"), originating from vesicular clusters that bud off the endoplasmic reticulum. A mammalian cell typically contains 40 to 100 stacks of cisternae.

Function:

The Golgi apparatus is a major collection and dispatch station of protein products received from the endoplasmic reticulum (ER). Proteins synthesized in the ER are packaged into vesicles, which then fuse with the Golgi apparatus. These cargo proteins are modified and destined for secretion via exocytosis or for use in the cell. In this respect, the Golgi can be thought of as similar to a post office: it packages and labels items which it then



Golgi body

sends to different parts of the cell or to the extracellular space. The Golgi apparatus is also involved in lipid transport and lysosome formation.

8. Lysosome:

Lysosome is an organelle in the cytoplasm of eukaryotic cells containing degradative enzymes enclosed in a membrane. Besides degradation of polymers, the lysosome is involved in various cell processes, including secretion, plasma membrane repair, cell signaling, and energy metabolism. [Settembre, C. et. al. 2013] Lysosomes are specialized vesicles within cells that digest large molecules through the use of hydrolytic enzymes. Vesicles are small spheres of fluid surrounded by a lipid bilayer membrane, and they have roles in transporting molecules within the cell.

9. Centrosome:

In cell biology, the centrosome (Latin centrum 'center' + Greek sōma 'body') is an organelle that serves as the main microtubule organizing center (MTOC) of the animal cell as well as a regulator of cell-cycle progression. [Bornens, M. 2008] In cell biology, the centrosome is an organelle that is the main place where cell microtubules are organized. Also, it regulates the cell division cycle, the stages which lead up to one cell dividing in two. Centrosomes are associated with the nuclear membrane during the prophase stage of the cell cycle. In mitosis the nuclear membrane breaks down and the centrosome nucleated microtubules can interact with the chromosomes to build the mitotic spindle.

MCQ - 1

1. Which of the following is the site of protein synthesis in a cell?

a) Mitochondria b) Ribosomes

c) Endoplasmic reticulum d) Nucleus

2. The powerhouses of the cell, which generate ATP, are known as:

a) Golgi apparatus b) Mitochondria

c) Lysosomes d) Vacuoles

3. The structure responsible for controlling what enters and leaves the cell is the:

a) Nucleus b) Plasma membrane

c) Mitochondrion d) Endoplasmic reticulum

4. Which organelle is responsible for modifying, sorting, and packaging proteins for secretion?

a) Nucleus b) Golgi apparatus

c) Lysosomes d) Ribosomes

5. The function of the smooth endoplasmic reticulum is primarily to:

a) Synthesize proteins b) Synthesize lipids

c) Modify proteins d) Produce ATP

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6. What is the function of the lysosome in animal cells?

a) Protein synthesis b) Cellular respiration

c) Digestion of cellular waste and debris d) Lipid synthesis

7. Which of the following organelles contains the genetic material in eukaryotic cells?

a) Nucleolus b) Ribosomes c) Nucleus d) Chloroplast

8. The double membrane structure that is involved in photosynthesis in plant cells is the:

a) Mitochondrion b) Chloroplast c) Ribosome d) Golgi apparatus

9. The function of the cytoskeleton is to:

a) Store energy b) Provide structural support and shape to the cell

c) Synthesizes proteins d) Modify and package proteins

10. Which of the following is a characteristic feature of prokaryotic cells?

a) Presence of a nucleus b) Membrane-bound organelles

c) A single circular DNA molecule d) Multicellularity

1.2. ORGANIZATION OF PROKARYOTIC AND EUKARYOTIC CELLS

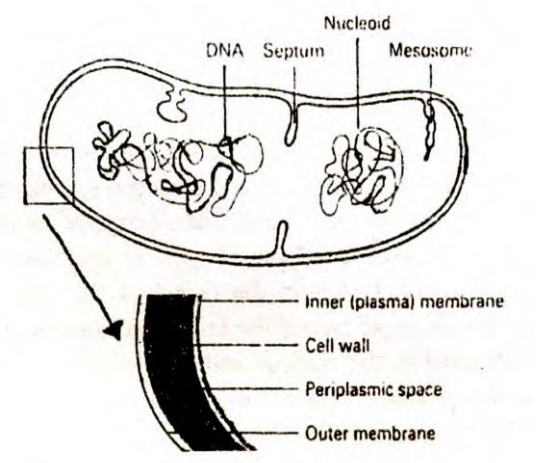
INTRODUCTION

Cells are the basic unit of life. In the modern world, they are the smallest known world that performs all of life's functions. All living organisms are either single cells, or are multicellular organisms composed of many cells working together.

Any cellular organism may contain only one type of cell from the following types of cells.

- **Prokaryotic cells**
- **Eukaryotic cells**

The terms prokaryotic and eukaryotic were suggested by Hans Ris in the 1960's.



1.2.1 PROKARYOTIC CELLS

In prokaryotic cells, in the absence of a nuclear envelope, the nuclear material is in direct contact with the cytoplasm. As an example, the structure of the colon bacterium, Eischerichia coli can be considered.

1.2.1.1. STRUCTURE OF PROKARYOTIC CELLS

Eischerichia coli can be easily cultured in a medium containing glucose solution and some minerals. In this medium, the number of individuals doubles within 60 minutes at 370C. If aminoacids, purines and primidines are added, the generation time can be reduced to only 20 minutes.

- A cell of E. coli is about 2m in length and 0.8m inthickness. A cell wall about 10nm in thickness surrounds the cell. It is made up of protein, polysaccharides inside the cell wall. The and lipid.
- A plasma membrane, lipoprotein, in nature lies inside the cell wall. This limits the cell volume and also regulates the movement of ions into and outside the cell. Enzymes constituting the respiratory chain and involved in the oxidation of metabolites are associated with the plasma membrane.
- The chromosomes of the bacteria are made up of a single circular molecule of deoxyribonucleic acid. This molecule, about 1mm. in length contains all the genetic information, and can code 2000 to 3000 different proteins.
- This DNA molecule is folded and lies free in the cytoplasm in the nuclear-region without any nuclear envelope. In the figure, DNA molecules are shown as the cell is about to divide and replication of DNA has occurred.

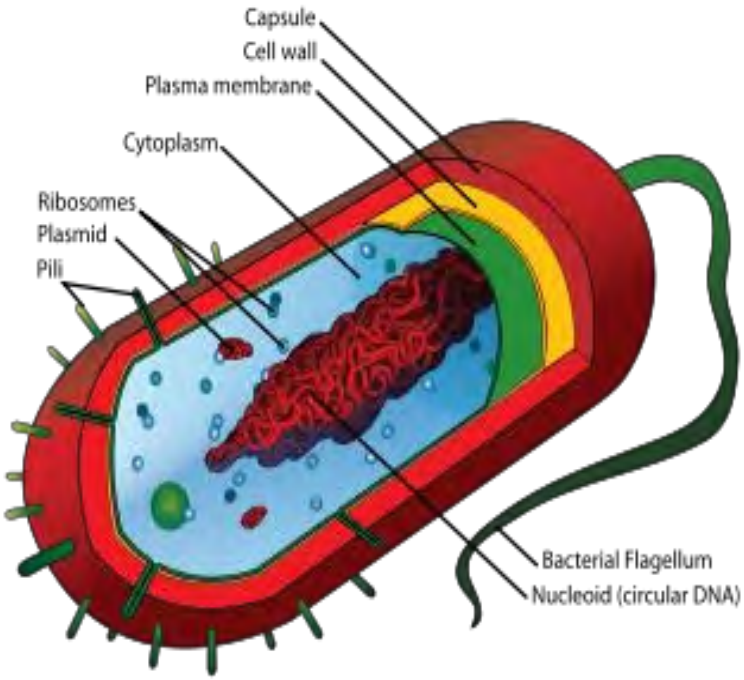
- Another characteristic feature membrane. The ribosomes consisting of RNA and proteins surround the DNA molecule and exist in groups called as the polyribosomes. These are made up of larger and smaller subunits. There are about 20,000 to 30,000 of them, each measuring about 25 mm in diameter.
- Water, protein molecules and various other types of molecules including RNA, fill up the remainder of the cytoplasm.
- The pleuropneumonia like organisms' range 0.1 to 0.25 m in diameter. These resemble in size the large sized viruses, such as the tobacco mosaic virus.
- The prokaryotic cells are generally smaller and vary in size in different members. In mycoplasma it is about 0.12 µm while in oscillatoria, a filamentous BGA the size is 40 x 5µ. A great majority of them, however are about smallest are to be found among cocci (0.1µ) while the largest are the spirilla (60 x 6µ).

BACTERIA

- Bacteria are unicellular organisms on an average, a cell range from 0.1µ to 1µ in size. Exceptionally some forms may be as large as 15 µ.
- Based on the cell shapes, four morphological forms have been identified in bacteria viz

Bacillus- The cells are rod shaped andelongated. There are second largest among bacteria

eg. Mycobacterium



Coccus

The cells are spherical. These are the smallest among bacteria.

Eg. Streptococcus, staphylococcus

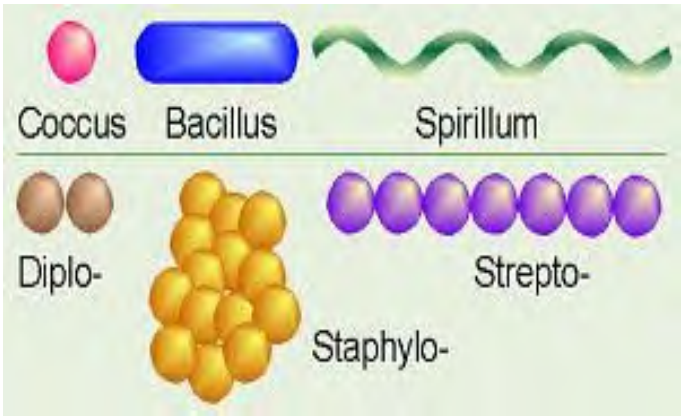
Spirillum

The cell will be in the form of a loose spiral. These forms are the largest.

Eg Rhodospirillum

Comma

- The cell will be in the form of punctuation mark, comma. Eg. Vibrio.
- Coccus form existing as single isolated cell it is called micrococcus, in pairs diplococcus, in chains streptococcus, in sheets staphylococcus.
- Many bacteria inhibit several shapes or forms. This is known as pleomorphism.



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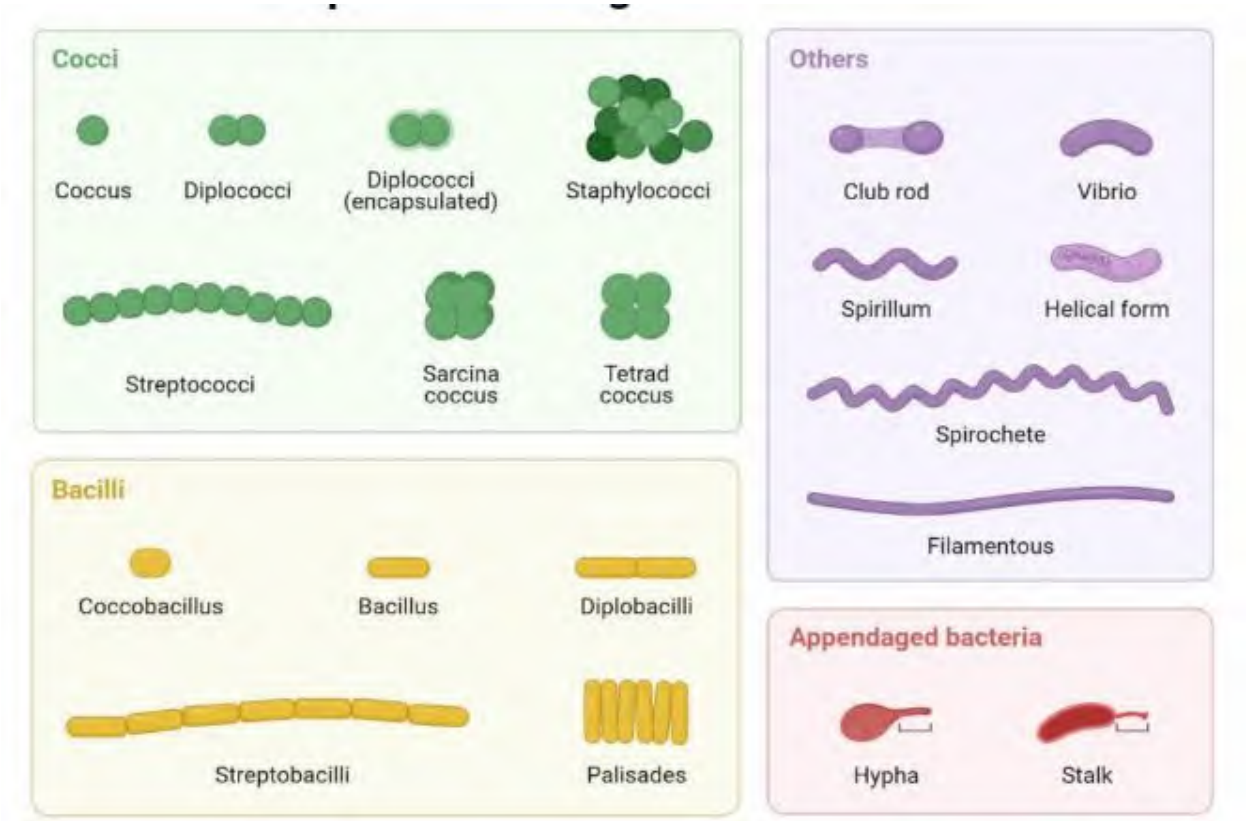
Locomotion

Except the coccus, all the others form of bacteria may possess organs of locomotion. ie. Flagella. The flagellum is a long thread like structures whose length usually exceeds that of the cell. It arises from a basal granule. On the basis of the number and position of flagella, bacterial forms are classified into

- Atrichous- No flagella. Eg. Cocci
- Monotrichous- Single flagellum present at one end eg. Vibrio
- Lophotrichous- Many flagella present at one pole (spirillum undula)
- Amphitrichous- Two tufts of flagella attached at either poles of the cell Eg. Spirilla

- Peritrichous- Flagella present all round Eg. Salmonella

Bacteria move with varied speeds. Some can move about 2,000 times their size in an hour. Hay bacillus can travel at a speed of 200 µ/s.



1.2.1.2 ULTRA STRUCTURE OF BACTERIA

Cell membrane

Internal to the cell wall is the cell membrane or the plasma membrane. It forms the outer boundary of the cytoplasm and is selectively permeable and it thus regulates the entry and exit of molecules into cytoplasm. The membrane is chemically made up of lipoproteins and practically no carbohydrates.

Outer covering

The outer covering of bacterial cell comprises the following 3 layers

- Plasma membrane
- Cell wall
- Capsule

Plasma membrane

- The bacterial protoplasts are bound by a living, ultra thin and dynamic plasma membrane.
- The plasma membrane chemically comprises molecules of lipids and proteins. Which are arranged in a fluid mosaic pattern.

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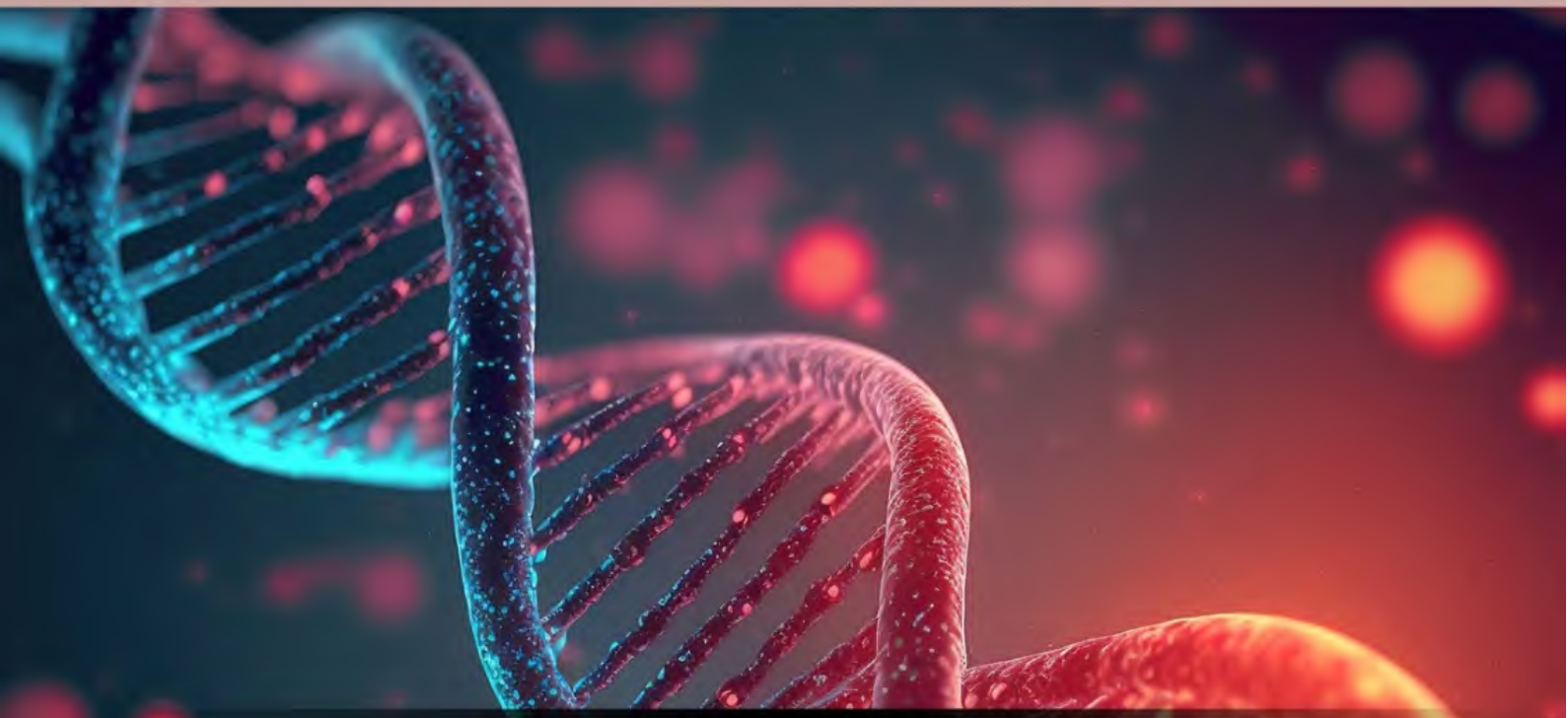

UNIT-VI

GENETICS AND BIOSTATISTICS & PLANT BREEDING

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GENETICS, BIOSTATISTICS AND PLANT BREEDING

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BOTANY - UNIT VI

GENETICS , BIOSTATISTICS AND PLANT BREEDING

SYLLABUS

GENETICS AND BIOSTATISTICS

Mandelian genetics - Mendel's Law of inheritance, non mendelian inheritance, Gene interactions - complementary genes, Lethal genes, Epistasis, Quantitative inheritance. Chromosomal basis of inheritance. Gene Linkage and crossing over - Kinds of linkage, types of crossing over mechanism. Sex determination in plants, theories of sex determination. Sex linked characters. Multiple alleles and pseudo alleles. Cytoplasmic inherritance, organelle heredity with reference to chloroplast and mitochondrial mutants -male sterility in plants. Population Genetics - Gene pool, Gene Frequencies, Mutation, selection, Migration, genetic drift, hardy - Weinberg law. Mutation, Selection, Migration, genetic drift, Hardy - Weinberg law. Mutation : Types of Mutation. Mutagenic agents and their mode of action.

PLANT BREEDING:

Domestication and introduction of plants. Origin of cultivated Plants. Vavilov's center of origin. Organic Agriculture. Conventional Plant Breeding systems: introduction , Selection - Types of selection, selection in self and cross pollinated crops. Hybridization-Hybridization techniques, male sterility, self - incompatibility, heterosis and hybrid vigor. Role of polyploidy in crop improvement. Green revolution, Applications of tissue culture and molecular techniques in plant breeding.

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MORE REFERENCE:

- PG TRB Previous Year Question Paper
- UGC NET Previous Year Question Paper

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UNIT VI – GENETICS , BIOSTATISTICS AND PLANT BREEDING

1. GENETICS

1.1 MENDELIAN GENETICS

- J**ohann Mendel** was the pioneer of classical geneticists. He was born in July 22,1822 in Heinzendorf in Austrian Silesia, where his father, **Anton Mendel** was the owner of a small farm. He graduated fr.om the Gymnasium in 1840.
- In his youth, he led a disastrous, poor, difficult and sad life. In October 1843, Mendel was admitted to the Augustinian monastery at Brunn in Moravia (a Czechoslovakian town) where he took the name **Gregor** as novice and besides performing his other duties, he took keen interest in natural sciences.
- In the year 1846, Johann Gregor Mendel attended courses of agriculture, pomiculture and viniculture at the Philosophical Academy in Brunn (now called **Brno** in Czechoslovakia).
- After finishing his theological studies in 1848, he was appointed as a substitute teacher in the Imperial Royan Gymnasium in Znaim in the year 1849. From 1851 to 1853, he studied mathematics and natural science in the university of Vienna. In 1853, he took the membership of Zoological-Botanical Society of Vienna.
- On April 5, 1854, he wrote a letter to Vienna Zoological-Botanical Society about damage by pea-weevil, *Bruchus pisi* and, thus, showed his interest in peas. In May 1854, he was appointed to the post of supply teacher of physics and natural science in a higher second dary school of Brunn.
- He continued to hold this post until 1868 when he was elected abbot. In the spring of 1856, he began experimental crossing of pea varieties. In 1862, Mendel became a founding member of the Brunn Natural Science Society.

- On February 8, 1865, he delivered his first lecture on pea experiments to Brunn Natural ScienceSociety. In 1866 his paper "*Experiments on plant hybridization"* published in volume 4 of the proceedings of the Natural Science Society.
- In the same year, he began experiments with other plant species. In this paper, Mendel proposed some basic genetic principles. But unfortunately his remarkable piece of work remained unattended and unappreciated up to 1900. There were several reasons for the sad neglect of Mendel's work.

These include

(i) biologist's preoccupation with speculation concerning Darwin's theory of evolution "origin of species" which appeared in 1859;

(ii) obscurity of the journal in which Mendel published his results; and

(iii) unaccoustomedness of professional biologists of ninteenth century to think in the statistical manner which Mendel introduced in the study of hybridization.

- Further, Mendel himself made his work known only to some of the most famous hybridizers of his time such as **Carl von Naegeli** and **Anton Kerner von Marilaum**, but not to the younger generation of scientists who were perhaps less prejudiced against new ideas.
- **Naegeli** and **Kerner** knew of the Mendel's paper but they did not review it or discuss it, perhaps because they considered him an outsider and amateur.
- Moreover, Naegeli's negative approach in discouraging Mendel to pursue the right path becomes apparent in his insistent suggestions to Mendel to test his genetical principles on 26 species of hawk-weed (*Hieracium*), for which he supplied seed and plants.
- When Mendel crossed many varieties of hawk-weeds, the progeny did not show evidence of segregation of genes from parents, but rather were all like their mothers. Later on, these plants were known to be apomictic (*i.e.,* parthenogenetic) or capable of reproduction without fertilization.
- Mendel wasted his valuable six years on the hybridization experiments on this plant species, ruined his eye sight, but even then failed to confirm or even to test his theory.
- The results of these ill-fated experiments were published in 1869, in the Proceedings of the Natural Science Society, Brunn. His defeat with *Hieracium* led Mendel to withhold further publication and eventually to cease scientific work.
- Further, this neglect of his work and other economical and physical hazards made him greatly disappointed and bitter.
- His health progressively degenerated and he became far too obese and began to suffer from dropsy due to heart and kidney failure. The pioneer of classical genetics, thus, died unknowingly, amidst the feelings of despair on 6th January 1884 and buried in Brunn Central Cemetery (See **Dunn**,1965; **Serra** 1965).

1.1.1 RE DISCOVERY OF MENDEL'S WORK

Mendel's research paper remained dormant and unnoticed by the scientific world until 1900. During these intervening thirty four years many developments occurred in biology which prepared the way for the rediscovery of Mendel's work. For instance, during this period **Haeckel** (1866) recognised the active role of nucleus in heredity; **Weismann**, **Hertwig**, **Strasburger** and **Kolliker** suspected active participation of chromosomes in heredity transmission. **Roux** (1883) suggested that the chromosomes must contain qualitatively different hereditary determiners arranged in linear orders. **Weismann** supported the idea of **Roux** by propounding his germplasm theory.

Further, some workers such as **Darwin** (1868) in England, **Vilmorin** (1879) in France, **Rumpau** (1891) in Germany and **Bohlin** (1897) in Sweden carried out hybridization experiments, very much like Mendel, on different plants and observed the phenomenon of dominance, but they failed to provide any conclusive explanation to their findings.

It was in the beginning of 20th century that three botanists, namely **Hugo de Vries,** working onc*Oenothera*; **Carl Correns** working on *Xenia,* peas and maize and **Erich von Tschermak** working oncvarious flowering plants, independently drawn the conclusions like Mendel. Later these botanists camecacross the research paper of Mendel and rediscovered it in 1900. Mendel's original paper wascrepublished in Flora, 89, 364 (1901). **Bateson** confirmed Mendel's work by a series of hybridization experiments.

1.1.2 MENDEL'S SELECTION OF THE EXPERIMENTAL PLANT

For his hybridization experiments Mendel had certain consideration in his mind about the choice of a suitable material. Mendel's considerations about the material were as follows :

1. Variation. The organisms which are to be chosen for the genetical experiments, should have a number of detectable differences and at a time only single detectable character should be considered.

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2. Reproduction. The chosen organisms should be sexually reproducing (*i.e.,* by fusion of male and female sex cells) because only then the offsprings will be able to receive different characters from both the male and female parents.

3. Controlled mating. The chosen organisms should be able to mate in controlled or well-planned conditions. Because in genetical experiments sometimes we have to rear genetically pure parents by methods of controlled mating. One should maintain careful records of the offsprings of many generation.

4. Short life cycle. The chosen organisms should have very short life cycles.

5. Large number of offsprings. The organisms which have been chosen for the genetical experiments should produce large number of offsprings after each successive mating because it will help in deducing the correct conclusions.

6. Convenience in handling. The experimental species should be of a type that can be raised and maintained conveniently and inexpensively in the laboratory. For instance, the elephant will prove entirely useless material for genetical experiments than the *Drosophila,* pea plants, tomato, rats, guinea pigs, etc., which have been generally used and are still used in hybridization experiments. *Arabidopsis thaliana* is a small, economically unimportant member of mustard family. In last four decades, it has became a most favorite research material for the plant geneticists and molecular biologists. *A. thaliana* is often nicknamed as *Drosophila melanogaster* (an insect) and *Caenorhabditis elegans* (a nematode) of the plant kingdom (**Gardner** *et al.,* 1991). This plant has the following advantages :

1. small size;

2. short generation time (about 5 weeks);

3. high seed production (up to 40,000 seeds per plant);

4. very small genome (7X107 nucleotide pairs);

5. very little interspersed repetitive DNA; and 6. natural self pollination.

1.1.3 MENDEL'S MATERIAL AND CROSSING TECHNIQUE

Mendel found edible pea (*Pisum sativum*) a best material for his hybridization experiments. The pea plant has various contrasting characters among its different varieties such as stem may be *tall* or *dwarf,* cotyledons may be *green* or *yellow*; seeds may be *round* or *wrinkled,* seed coat may be *coloured* or *colourless*; the unripe pods may be *green* or *yellow*; the ripe pods may be *inflated* or *constricted* between the seeds, flowers may have *axial* or *terminal* positions and the colours of flowers may be *red* or *white.* Besides these contrasting characters, the pea plant is a very satisfactory material for the hybridization experiments due to its flower structure.

The flowers of pea plants are so constructed that the pollens of a flower normally fall on the stigma of the same flower and, thus, affects self-pollination or self-fertilization. For the required cross-pollination, the anthers have to be removed from the flower in bud stage (*i.e.,* before their maturity). This operation of removal of anthers is called **emasculation**.

The stigma is protected against any foreign pollen with the help of its covering by a bag. The pollen, then at the dehiscence stage is brought from the plant to be used as a male parent and by the help of a brush is dusted on the feathery stigma of the **emasculated** flower. At the time of such **cross pollination**, the pollen should be mature and stigma should be receptive.

For each of seven pairs of characters plants with one alternative trait were used as female, and those with the other alternative as male. Reciprocal crosses were also made, *i.e.,* each of the crosses was made in two ways, depending on which phenotype is used as male or female. For example the following two crosses are reciprocal crosses :

phenotype A (*e.g.,* Tall) & × phenotype B (*e.g.,* Dwarf) %

phenotype B (*e.g.,* Dwarf) & × phenotype A (*e.g.,* Tall) %

The population obtained as a result of crossing plants showing contrasting characters is called **F1 generation**. The progeny of F1 plants was then obtained by self-fertilization and it forms the **F2 generation**. Similarly, F3, F4, etc., generations can also be obtained.

Further, for getting the exact results in the breeding experiments, it was necessary for Mendel to rear genetically pure variety of pea plants for a single character. Mendel adopted self-fertilization technique for it. For instance, to get pure character for tallness, he self fertilized a tall pea plant for many generations till the resulted offsprings always produced only tall plants.

Likewise, he got genetically pure variety for dwarf pea plants. Mendel cross pollinated these two varieties of pea plants which were differing in a pair of contrasting characters, *viz.,* tallness and dwarfness of the stem. When he made observations on the offsprings of first generation he found only tall plants. He allowed the self pollination in the offsprings of first generation and made further observations on the offsprings of second generation. He was astonished to note both tall and dwarf offsprings in the second generation.

This showed him that the character of dwarfness disappeared in first generation but again reappeared in second generation. Further, the tall and dwarf plants of second generation were always in the ratio of 3:1 (3 tall : 1 dwarf). He self-pollinated the dwarf offsprings of second generation and found only dwarf plants in third generation. But when he self-pollinated the tall plants of second generation then he found that onethird (1/3) tall plants yield only tall plants in third generation, while rest two-third (2/3) tall plants yield tall and dwarf plants in the ratio of 3:1. On the basis of the results of his experiments Mendel recognized the **phenomenon of dominance** and formulated following two laws :

1. Law of segregation ;
2. Law of independent assortment.

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Actually Mendel himself did not postulated any genetical principle or laws, he simply gave conclusive theoretical and statistical explanations for his hybridization experiments in his research paper. However, it was **Correns**, the discoverer of Mendel's work, who thought that Mendel's discovery could be represented by these fundamental laws of heredity.

1.1.4 PHENOMENON OF DOMINANCE

The cross between the pea plants differing in single pair of contrasting characters is known as **monohybrid cross**. As we have already noticed that when Mendel made a monohybrid cross between tall and dwarf pea plants then only tall pea plants appeared in the first filial generation (F1). But when the F1 progeny were allowed to be self-fertilized, both tall and dwarf characters appeared in the second filial generation or F2. This shows

that in F1 hybrid the character of tallness dominates or conceals the character of dwarfness and so the character of dwarfness could not express itself in F1 generation. The character which expresses itself (*i.e.,* tall) in F1 generation is called by Mendel as **dominant character**, while the character which remained unexpressed or latent had been called **recessive**.

According to these results Mendel described the phenomenon of dominance in following way : in crossing between pure (homozygous) organisms for contrasting characters of a pair, only one character of the pair appears in the first filial generation. In pea plant Mendel found following characters to be dominant or recessive in various pairs of contrasting characters.

Certain Examples of Phenomenon of Dominance

After Mendel several geneticists tested the validity of the phenomenon of dominance on several plants and animals. They found its wide application in various plants and animals. A few important examples are described as follows :

1. Phenomenon of Dominance in Plants

Besides pea plant the phenomenon of dominance has also been observed in the following plants.

2. Application of Phenomenon of Dominance in Animals

The phenomenon of dominance is also applicable well to the animals. For instance, when homozygous black guinea pig is crossed with a homozygous white guinea pig (then all hybrids of first filial generation (F1) are found to be black. The black hybrids of F1 when mated among themselves they produced black and white offsprings in 3:1 ratio. This shows that black coat colour dominates over white coat colour.

Actual data obtained by Mendel in his monohybrid crosses and certain modern data.

Character studied	Old and recent symbols	Current symbols	Chromosome location*	Appearance of all F_1 hybrids	Appearance of F_2 plants	F_2 ratio
1. Seed form : Round × Wrinkled or Rugosus	R, r ; W, w	R, r	7	Round	5474 Round 1850 Wrinkled or Rugosus	2.96 : 1
2. Cotyledon colour : Yellow × Green	Y, y ; G, g	I, i	1	Yellow	6022 Yellow 2001 Green	3.01 : 1
3. Seed coat colour : Grey × White	G, g; W, w	A, a	1	Grey	705 Grey 224 White	3.15 : 1
4. Pod form : Inflated × Constricted	I, i; C, c	V, v	4	Inflated	882 Inflated 299 Constricted	2.95 : 1
5. Pod colour : Green × Yellow	G, g; Y, y	Gp, gp	5	Green	428 Green 152 Yellow	2.82 : 1
6. Flower position : Axial × Terminal	A, a; T, t	Fa, fa	4	Axial	651 Axial 207 Terminal	3.14 : 1
7. Stem length : Tall × Dwarf or Short	T, t; D, d	Le, le	4	Tall	787 Tall 277 Dwarf	2.84 : 1

The dominant and recessive characters in plants.

Name of the plant	Dominant	Recessive
1. Nettle	Serrated leaves	Smooth margined leaves
2. Sunflower	Branched habit	Unbranched habit
3. Cotton	Coloured lint	White lint
4. Maize	Round starchy kernel	Wrinkled sugary kernel
5. Snapdragon	Red flower	Non-red flower
6. Barley	Beardlessness	Beardness
7. Wheat	Susceptibility to rust	Immunity to rust
8. Tomato	Two celled fruit	Many celled fruit

B. Certain other examples of phenomenon of dominance in animals. The dominant and recessive traits or characters of some animals can be tabulated in following table :

The dominant and recessive characters in animals.

Name of animal	Body character	Dominant	Recessive
1. Cat	Skin colour	Tabby	Black or blue
	Length of hair	Short hairs	Long hairs (Angora)
2. Dog	Skin colour	Grey	Black
	Tail	Stumpy	Normal tail
3. Cattle	Colour of face	White	Coloured
	Horn	Polled or Hornless	Horned
4. Horse	Skin colour	Black	Red
	Movement	Trotting	Pacing
5. Sheep	Hair or wool or fleece	White	Black
6. Swine or Pig	Skin colour	Black	Red
	Hoof	Uncleft	Normal
7. Salamander	Body colour	Dark	Light
8. Fruit fly (*Drosophila*)	Eye colour	Red	White
	Wings	Flat and yellow	Curled and white
	Body colour	Grey	Black
9. Land snail	Shape of shell	Unbanded shell	Banded shell

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1.1.5 LAW OF DOMINANCE

When Mendel crossed a true breeding yellow seeded plant with a true breeding green seeded plant, it resulted in the production of only yellow seeded offspring in the Fl generation green colour was suppressed and the yellow colour exhibited dominance. Mondel described the characters such as yellow dominant and their alternative characters such as green, recessive. All other paired characters studied by Mendel exhibited similar dominance.

1.1.6 LAW OF SEGREGATION:

Mendels monohybrid cross - cross between homozygous yellow seeded plant and homozygous green seeded plant - produced all yellow seeded plants in F1 generation. Yellow character is considered dominant over green. When F1 plants were crossed among themselves or fertilized, in the F2 generation three-fourths of the plants. (6022 out of 8023)

resembled the dominant grand parent and one-fourth (2001 out of 8023) resembled the recessive grand parent. These results led Mendel to the discovery of the law of segregation.

Let the genotype of the pure yellow seeded plant beassumed YY as it contains two yellow genes in its body cells. The plant produces only one type of gametes with a Y (yellow) gene. Let the genotype of the pure green seeded plant be yy as it contains two green genes in its body cells. This plant also produces only one type of gametes but with a y (green) gene. A cross of these two plants results in the production of only one kind plants in the F1 generation with the genotype Yy. All F1generation plants are yellow seeded because Y gene (yellow) is dominant over y gene (green).

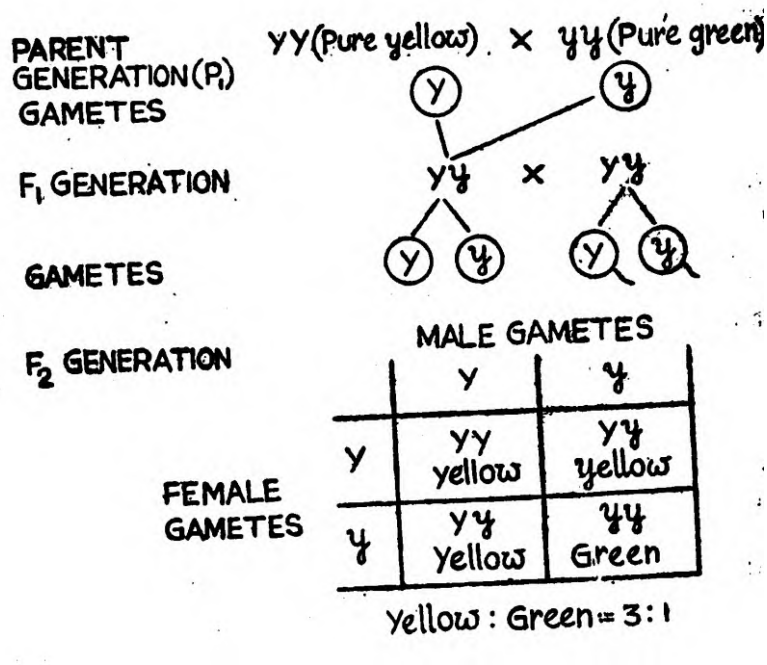
In the hybrid plant of F1 generation, the yellow and green genes, though they are intimately associated, do not mix or pollute each other. Moreover these genes separate or segregate and enter different gametes of the F1 plant and finally reach different individuals of the F2 generation. Mendel was forced to come to this conclusion from the results of his breeding experiment.

In a cross between two F1 hybrid plants, each one produces to types of gametes - gametes with Y (Yellow) gene and gametes with y (green) gene in equal numbers. Random mating of the gametes results in three types of genotypes in the F2 generation with predictable ratios i.e. 25 percent YY, 50 percent Yy and 25 percent yy. The external expression of this generation will be 75 percent yelllow and 25 percent green.

In other words the phenotypic ratio of monohybrid cross is 3:1 and the genotypic ratio is 1:2:1. The cross can be summed up as in. Mendel postulated a general rule from the results of his mohybrid cross. The two members of each pair of genes must separate when gametes are formed and only one of each pair can go to a gamete. This is the law of segregetion. It is applicable to the paired genes that are identical (YY or yy - homozygous) or dissimilar genes (Yy - heterozygous).

1.1.7 TEST CROSS :

Mendel verified his law of segregation by conducting a test cross or back cross. He crossed F1 hybrid yellow seeded plant with the recessive green seeded plant. The offspring of this cross can be predicted by assuming the segregation of genes. F1 hybrid plant (Yy) produces Y gametes and y gametes in equal numbers. The green plant produces only one kind of gametes with a y gene. The offspring of this test cross must be, if Mendels theory of segregation is correct, 50 percent yellow (Yy) coloured seed plants and 50 percent green (yy) coloured seed plants. In otherwords the back cross ratio of tha monohybrid cross is 1:1. Thousands of test crosses conducted by Mendel and others produced 1: 1 ratio in the offspring.



The homozygous long-winged Vinegar-fiy crossed with a homozygous vestigial winged one produces all long winged hybrid individuals in F1 generation. The crosses between the F1 individuals resulted in the segregation of long winged and vestigial-winged flies in the ratio of 3:1 in the F2 generation.

(Examples from animals:

2. Andalusian fowls:

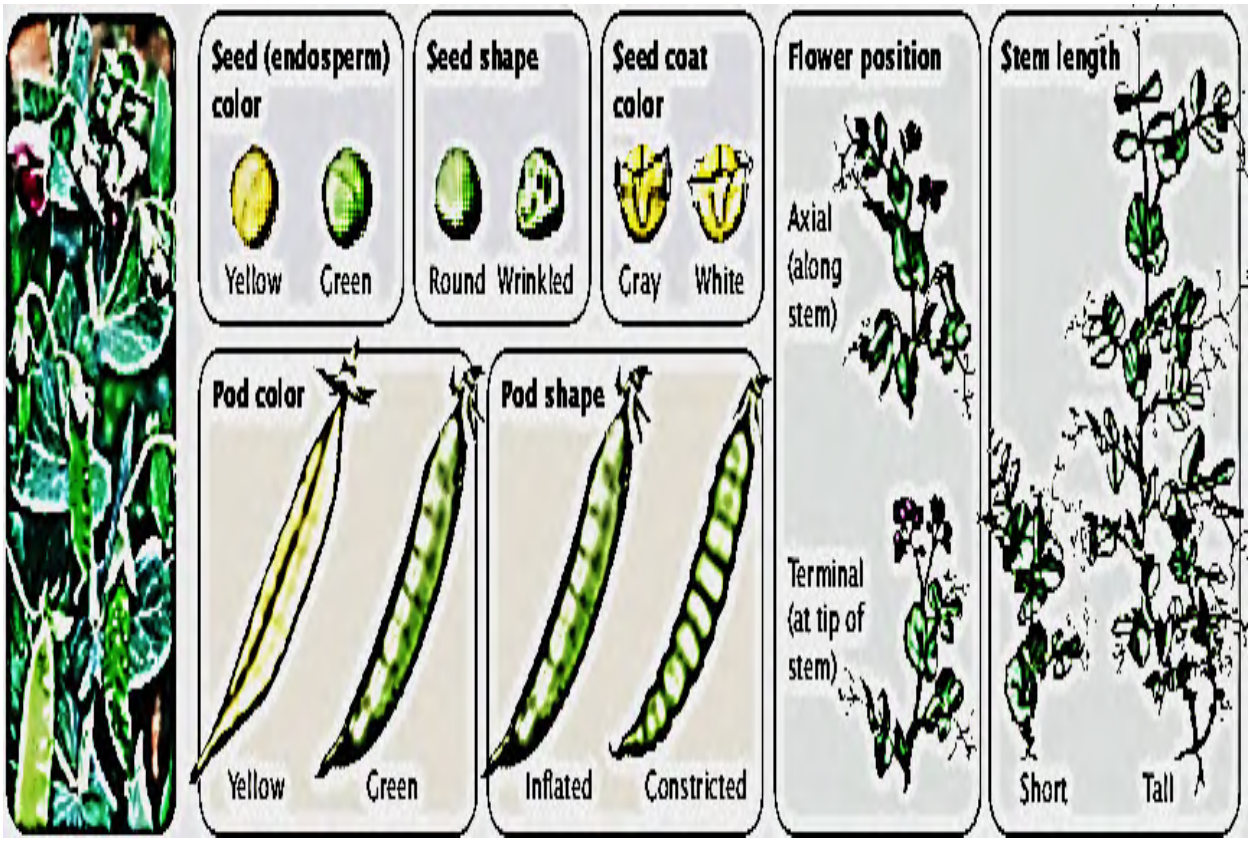
The colour of the plumage in these fowls is inherited on the principle of segregation. Interesting dominance is absent in this cross. A black fowl crossed with a white one yielded all blue plumage offspring in the F1 generation. Crosses of F1 individuals segregated the black, blue and white birds in the ratio of 1:2:1. In this example the phenotypic ration of monohybrid cross black : blue : white is 1:2:1 and not 3:1. This is due to lack of dominance.)

1.1.8 LAW OF INDEPENDENT ASSORTMENT:

Mendel presented the results of his experiments at two meetings (8th February and 8th March, 1865) of the Brunn Natural History Society. No one who heard or read the Mendel's paper in the 19th century recognised the importance of his work. It was neglected until the ye ir 1900 when De Vries in Hollal, Correns in Germany and Tshermark in Austria rediscovered simultaneously by obtaining results similar to those of Mendels from their own breeding experiments. Mendel died in 1884.

Gregor Johann Mendel was born in 1822 to a peasant family in the central European village of Heinzendorf. An excellent student in high school, he studied philosophy for several years after-ward, and in 1843 he was admitted to the Augustinian Monastery of St. Thomas in Brno, now part of the Czech Republic, taking the name Gregor. In 1856, Mendel performed his first set of hybridization experiments with the garden pea (*Pisum sativum)*.

The research phase of his career lasted until 1868, when he was elected abbot of the monastery. Although he retained his interest in genetics, his new responsibilities demanded most of his time.



He chose an organism that is easy to grow and hybridize artificially. The pea plant is self-fertilizing in nature but is easy to crossbreed experimentaly. It reproduces well and grows to maturity in a single season. Mendel followed seven visible features (unit characters), each represented by two contrasting forms, or traits. For the character stem height, for example, he experimented with the traits tall and dwarf.

He selected **six** other visibly **contrasting pairs of traits** involving

1. Seed shape
2. Seed color
3. Pod shape
4. Pod color
5. Flower position
6. Stem length

1.1.9 LAWS IN CROSSING TECHNIQUES

Crosses involving inheritance of only one pair of contrasting characters are called monohybrid crosses and those involving two pairs of contrasting characters are called dihybrid crosses.

Mendel performed monohybrid, dihybrid and polyhybrid crosses and formulated basic laws of heredity. These laws are:

Law of dominance

When two factors (now known as genes) of a pair of contrasting characters are brought together in a cross, only one of them express itself in the resulting hybrids. The character expressed is said to be dominant and the other that remains suppressed is called recessive.

Law of segregation

Each organism contains 2 factors for each trait, and the factors segregate during the formation of gametes so that each gamete contains only one factor for each trait. When fertilization occurs, the new organism has 2 factors for each trait, one from each parent.

Law of independent assortment

When the factors (genes) for different characters inherited from parents**.(PG TRB 2003-2004)**, do not remain linked in the offspring, but their distribution in the gametes and in the progeny of subsequent generations is independent of each other. The characters, which follow the Mendel's laws during inheritance, are called Mendelian traits.

Incomplete Dominance

Sometimes in a heterozygote dominant allele does not completely mask the phenotypic expression of the recessive allele and there occurs an intermediate phenotype in the heterozygote. This is called incomplete dominance.

Codominance

Sometimes both alleles of a gene in a heterozygote lack the dominant and recessive relationship, ie., each allele is capable of some degree of phenotypic expression. In a sense, codominance is no dominance at all, the heterozygote showing the phenotypes of both homozygotes. Hence, heterzygote genotype gives rise to a phenotype distinctly different from either of the homozygous genotypes.

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1.1.10 MONOHYBRID CROSS

Modern Genetic Terminology

To illustrate the monohybrid cross and Mendel's first three postulates, we must first introduce several new terms as well as a symbol convention for the unit factors.

Traits such as tall or dwarf are visible expressions of the information contained in unit factors. The physical appearance of a trait is the **phenotype** of the individual. Mendel's unit factors represent units of inheritance called **genes** by modern genetics.

For any given character, such as plant height, the phenotype is determined by alternative forms of a single gene called **alleles**. **.(PG TRB 2002-2003)**

Alternative genes controlling characters are called alleles. For example, the unit factors representing tall and dwarf are alleles determining the height of the pea plant.

Term	Definition
Gene	A genetic factor (region of DNA) that helps determine a characteristic
Allele	One of two or more alternate forms of a gene
Locus	Specific place on a chromosome occupied by an allele
Genotype	Set of alleles that an individual possesses
Heterozygote	An individual possessing two different alleles at a locus
Homozygote	An individual possessing two of the same alleles at a locus
Phenotype or trait	The appearance or manifestation of a character
Character or characteristic	An attribute or feature

Thus, for Mendel's pea plants, we used trait based on stem length for the '**d**' dwarf allele and '**D**' for the tall allele. When alleles are written in pairs to represent the two unit factors present in any individual (DD, Dd, or dd), these symbols are called the **genotype.(PG TRB 2001)**

- When a gene pair in an organism contains two identical alleles eg., D and d, the organism considered homozygous for that gene pair and is called **homozygote (DD, dd)**.
- When two different alleles are present in a single gene pair eg., D and d the organism is heterozygous for that gene pair and is called **heterozygote (Dd).**

In each monohybrid cross, the trait expressed in the F1 generation is controlled by the **dominant** unit factor. The trait not expressed is controlled by the **recessive** unit factor. The terms dominant and recessive are also used to designate traits. In this case, tall stems are said to be dominant over the recessive dwarf stems. The genotypes and phenotypes resulting from the recombination of gametes during fertilization can be easily visualized by constructing a **Punnett square**, named after the person who first devised this approach, **Reginald C. Punnett**. L. Monohybrid cross is based on the **law ofsegregation**.

In monohybrid cross trait is based on stem length (tall and dwarf). Tall (DD) and dwarf (dd) plant were crossed as result in the first generation F1 all plants were tall (Dd) but they are in heterozygous of single trait in nature. During F2 generation, cross of two F1 hybrids, heterozygous for a single trait that displays incomplete dominance is predicted to give a 1:2:1 ratio among both the genotypes and phenotypes of the offspring. 1/4 will have the dominant phenotype (tall, DD), 1/2 will have the intermediate phenotype (Dd) resembling the parents of this cross, and 1/4 will have the recessive phenotype (short, dd). Note the case with which the monohybrid **phenotypic ratio is 3:1 (tall:dwarf)** and the **genotypic ratio is 1:2:1** (DD, Dd, dd) derived in the F2 generation.

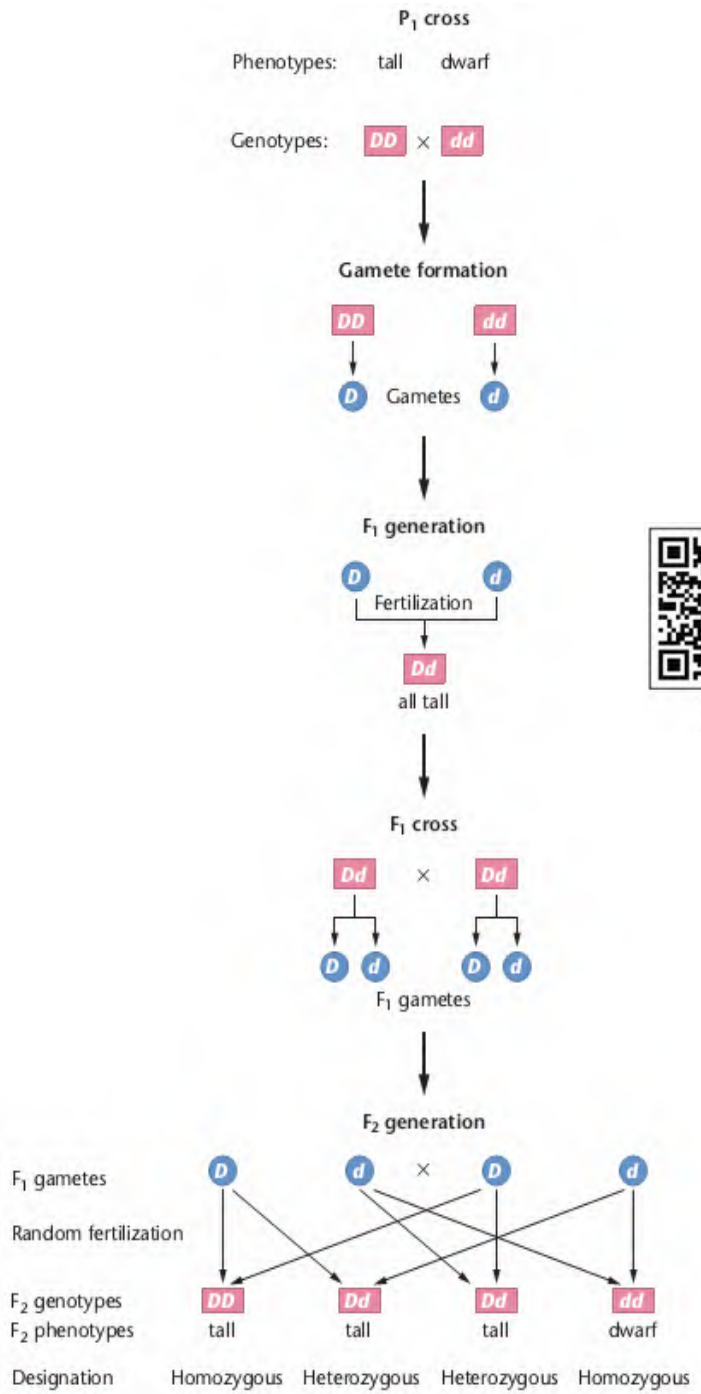

FIGURE 3-2 The monohybrid cross between tall (*D*) and dwarf (*d*) pea plants. Individuals are shown in rectangles, and gametes in circles.

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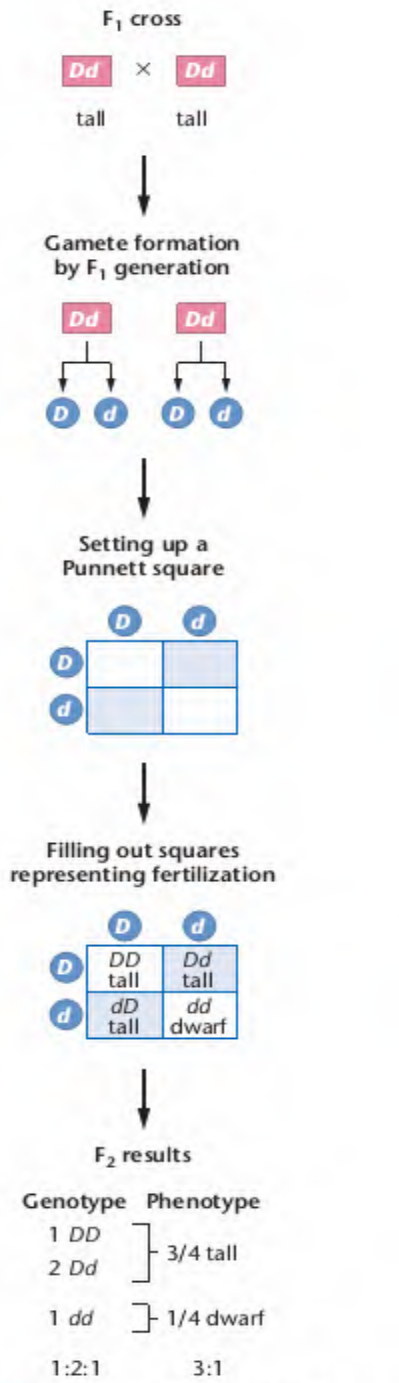

FIGURE 3-3 A Punnett square generating the F_2 ratio of the $F_1 \times F_1$ cross shown in Figure 3–2.

1.1.10.1 BACK CROSS AND TEST CROSS

Test cross is a simple method devised by Mendel to verify the genotype of the F_1 hybrid. When the F_1 hybrid is crossed with the homozygous recessive parent, it is called a **test cross**. **.(PG TRB 2017)** Since, the F_1 is crossed back with one of the parents, it is also called a **back cross**.

Test cross is also used for checking the correctness of Mendel's law of segregation (using a monohybrid test cross) and the law of independent assortment of characters (using a dihybrid test cross).

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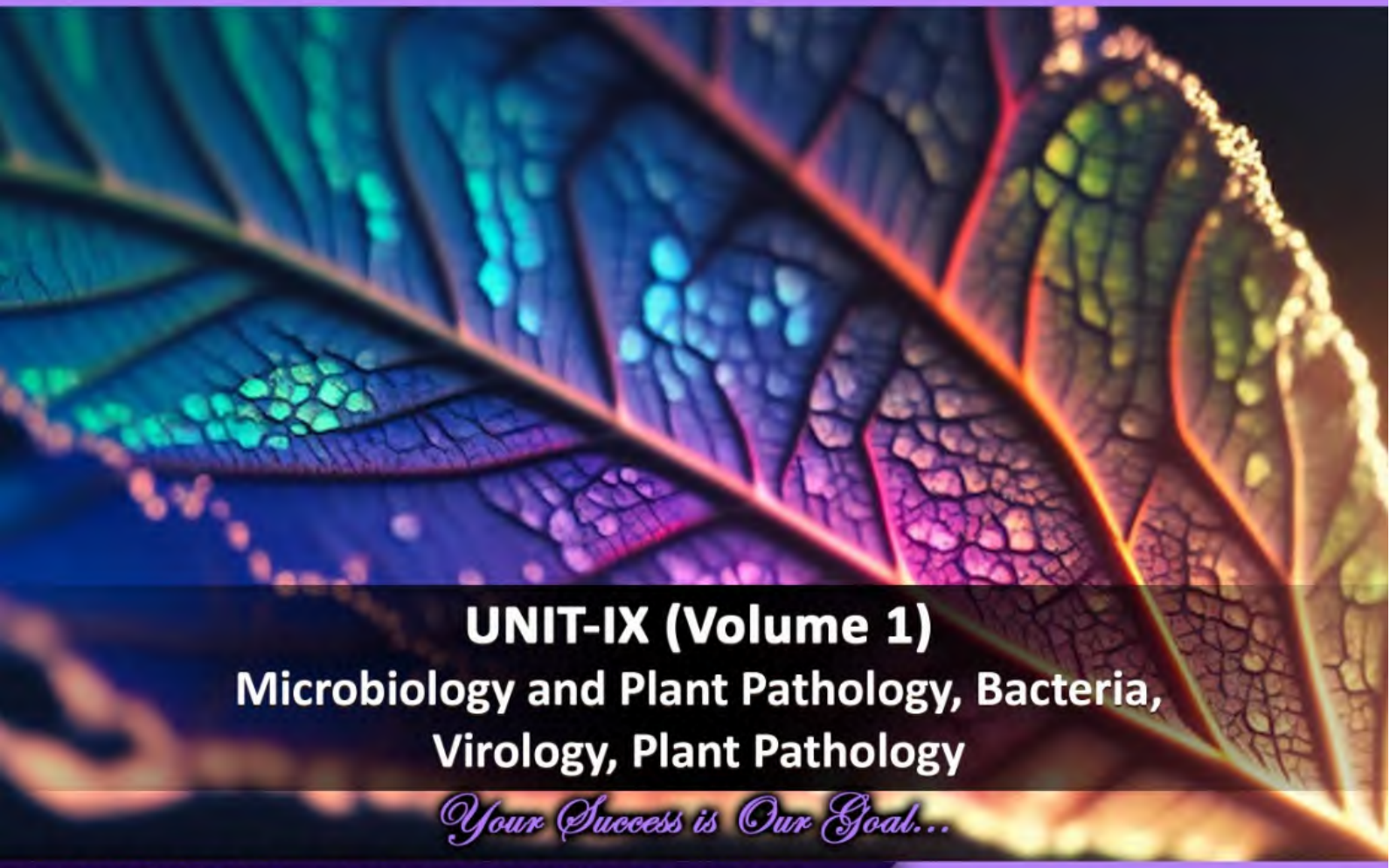

UNIT-IX (Volume 1)

Microbiology and Plant Pathology, Bacteria, Virology, Plant Pathology

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UNIT – 9 (VOL-1)

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SYLLABUS

MICROBIOLOGY AND PLANT PATHOLOGY

BACTERIA:

Classification of bacteria (Bergey's Manual of Bacteriology1994), structural organization and reproduction of bacteria, Motility, flagella and pili – Growth and Nutrition, growth curve, kinetics of bacterial growth. Sterilization techniques, culture media, staining techniques for bacterial identification – Bacterial genetics: conjugation, transformation and transduction. Application: fermentor and types of fermentations – industrial products from bacteria, agricultural applications of bacteria, bacteria in Bioremediation. Structure and reproduction of Archaebacteria, Cyanobacteria, Mycoplasma and Actinobacteria (Actinomycetes).

VIROLOGY:

General characteristics, Classification of plant viruses (ICTV,1970), structure and multiplication of plant viruses. Bacteriophage: Structural characteristics and multiplication. Virion, viroid, virusoids and prions. Isolation and purification of plant viruses.

PLANT PATHOLOGY:

Classification of plant diseases, Symptomology. Principles of plant infection: Inoculum, inoculum potential, Pathogenicity, Disease triangle. Epidemiology and forecasting of plant diseases – Host parasite inter relationships and interaction. Pathogenesis: Mechanism of penetration- Disease development of pathogen (colonization) and dissemination of pathogens. Environment and nutrition in relation to disease development – Defence mechanism. Role of enzymes and toxins in disease development. Diseases and disease cycle – Important diseases of crop plants in India: Sheath blight of rice, leaf spot of groundnut, Black rust of wheat, Late blight of potato, Fusarium wilt of cotton, Bacterial blight of rice, Citrus canker, Bunchy top of Banana, Root knot of Brinjal, Red rust of tea. Disease Resistance mechanism in plants. Techniques adopted in plant breeding for disease resistance. Principles of plant disease management – Cultural practices, physical, chemical and biological methods, disease controlled by immunization. Plant quarantine and legislation. Integrated Pest Management System. Plant protection organizations in India.

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9. Plant Pathology - By Dr. P.D. Sharma

10. Microbiology & Plant Pathology - By Dr. P.D. Sharma

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UNIT – IX (VOL-1)

MICROBIOLOGY AND PLANT PATHOLOGY

1.BACTERIA

INTRODUCTION

- Bacteria are microscopic living organisms that have only one cell. The word for just one is "bacterium." Millions (if not billions) of different types of bacteria can be found all over the world, including in your body.
- They're on your skin and in your airways and mouth. They're also in your **digestive system,** reproductive system and urinary tract.
- Scientists estimate you have 10 times more bacterial cells than human cells in your body.

1.1 BERGEYS CLASSIFICATION OFBACTERIA 1994

- Bergey's Manual of Systematic Bacteriology is a key resource for identifying and classifying bacteria.
- The 1994 edition refers to the second edition of **Bergey's Manual of Systematic Bacteriology**, which was published in volumes between 1984 and 1989.
- This second edition was significant in providing a comprehensive, systematic approach to bacterial classification based on both phenotypic and genotypic characteristics.
- The 1994 edition, while not specifically released in that year, represented the culmination of work from earlier volumes and is often associated with the following updates.

1.1.1 Key Elements of Bergey's Manual of Systematic Bacteriology (2nd Edition):

1. **Phylogenetic Approach**:

- The classification in the 2nd edition of Bergey's Manual emphasized phylogenetic

relationships, meaning the evolutionary history and genetic lineage of the organisms. It was a shift away from previous phenotypic classifications.

- This allowed for more accurate groupings of bacteria based on shared genetic traits, rather than just appearance and biochemical characteristics.
- The **phylogenetic approach** introduced in the **2nd edition of Bergey's Manual of Systematic Bacteriology** represents a fundamental shift in how bacteria are classified and categorized.
- Prior to this, bacterial classification was primarily based on phenotypic characteristics, such as morphology, biochemical properties, and Gram-staining patterns. However, with advancements in molecular biology, particularly the discovery of **genetic sequences** that provide deeper insights into evolutionary relationships, Bergey's Manual began to emphasize **phylogeny**—the evolutionary history and relationships among organisms.

Here's a detailed breakdown of the **phylogenetic approach** in the 2nd edition:

A. DNA-Based Classification

- One of the cornerstones of the phylogenetic approach is the use of **molecular markers**, such as the **16S rRNA gene**. The 16S ribosomal RNA gene is highly conserved across bacteria and is often used as a molecular fingerprint to establish evolutionary relationships among bacterial species.
- This gene is part of the ribosome, the molecular machinery responsible for protein synthesis, and its sequence is considered to evolve at a relatively slow pace, making it useful for determining the phylogeny of bacteria.
- **16S rRNA Gene Sequencing**: The sequencing of this gene allows for comparisons of genetic material across different bacteria, revealing their evolutionary relatedness.
- **Molecular Phylogenetics**: By comparing 16S rRNA gene sequences, scientists can build **phylogenetic trees** that show how different bacteria are related, forming a **molecular phylogeny**.

B. Ribosomal RNA as a Key Characteristic

- The 16S rRNA gene is used to infer the **phylogenetic tree of life**. The sequence of this gene in various bacterial species can be compared to determine how closely related they are.
- The phylogenetic tree is structured in a way that groups organisms based on shared ancestry, helping taxonomists better understand their evolutionary relationships.
- In the **2nd edition of Bergey's Manual**, the use of **16S rRNA gene sequencing** and other molecular techniques became a significant method for defining bacterial taxa. This is in contrast to earlier editions, where phenotypic characteristics (like shape, size, staining patterns, and biochemical properties) were the primary tools for classification.

C. Revised Taxonomic Hierarchy

- As a result of applying phylogenetic principles, many bacterial groups were reorganized based on their genetic relationships. Some bacterial groups that were once thought to be distantly related were found to be more closely related than previously believed, while others that appeared similar morphologically were found to belong to different evolutionary lineages.
- **Phyla**: The second edition classified bacteria into several major groups or **phyla** (plural of phylum), such as:

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- **Proteobacteria**
- **Bacteroidetes**
- **Firmicutes**
- **Chloroflexi**
- **Actinobacteria**
- **Tenericutes** (including Mycoplasmas)
- These phyla were based on **genetic similarities** and evolutionary relationships, not just morphological traits.
- **D. The Rise of the Three-Domain System**
- In line with the phylogenetic approach, the second edition of Bergey's Manual reflected the growing acceptance of the **three-domain system of life**:
- **Bacteria** (prokaryotes, typically single-celled organisms)
- **Archaea** (extremophiles, previously classified as bacteria, but now recognized as a separate domain based on genetic differences)
- **Eukarya** (organisms with complex cells, including plants, animals, fungi, etc.)
- This three-domain system emphasized the evolutionary distance between the Archaea and Bacteria, acknowledging their distinct genetic lineages. The **Archaea** were recognized for their unique genetic and biochemical characteristics that set them apart from bacteria, despite their similar outward appearance.

E. Genetic Markers and Cladistics

- The phylogenetic approach also brought **cladistics** to the forefront of bacterial classification.
- Cladistics is a method of grouping organisms based on common ancestry. It uses shared derived traits (known as **synapomorphies**) to build a **cladogram** or tree that represents evolutionary relationships.

For instance:

- **Proteobacteria** and **Firmicutes** are two major groups of bacteria, but through genetic analysis, we now know that they diverged from a common ancestor far earlier than was previously assumed by phenotypic methods.
- **The use of genetic markers** (other than 16S rRNA) like **housekeeping genes** also

came into play for further subclassifications and understanding of phylogenetic relationships.

F. Impact on Bacterial Taxonomy

The phylogenetic approach radically changed the way bacterial taxonomy was understood:

1. **Species Classification**: Bacteria that were once grouped together because they shared similar biochemical or morphological characteristics were sometimes reclassified into entirely different taxonomic categories once their genetic relationships were understood.
2. **Revised Nomenclature**: The classification system was updated to reflect these molecular discoveries. Species that were once thought to be similar (e.g., due to similar shapes or biochemical properties) might now be recognized as genetically distinct, leading to a reorganization of bacterial families, genera, and species.

G. Incorporation of New Taxa and Extant Phyla

With the phylogenetic approach, Bergey's Manual also updated the taxonomy to reflect new discoveries:

- **Archaea**: The previously grouped Archaea and Bacteria were now clearly separated, with Archaea forming their own distinct domain.
- New species and genera were classified more accurately based on molecular data, even if they had previously been misclassified due to phenotypic similarities to other groups.

2. **Five Major Groups**: The bacteria were grouped into five major categories, or "phyla," based on their molecular and genetic similarities:

- **Gracilicutes** (Gram-negative bacteria)
- **Firmicutes** (Gram-positive bacteria)
- **Tenericutes** (Mycoplasmas, bacteria without a cell wall)
- **Mendosicutes** (Archaea, extremophiles and methanogens)
- **Scotobacteria** (An older classification which, in the 2nd edition, was not used in the same form as newer groups)

3. **Volume-Based Classification**: The second edition was organized into a series of volumes, each dedicated to specific types or groups of bacteria:

- Volume 1: **The Archaea and the Deep-Branching and Phototrophic Bacteria**
- Volume 2: **The Proteobacteria (Alpha, Beta, Gamma, Delta, and Epsilon Subdivisions)**
- Volume 3: **The Firmicutes and Tenericutes**
- Volume 4: **The Actinobacteria and other Gram-Positive Bacteria**
- Volume 5: **The Bacteroidetes, Planctomycetes, and other Gram-Negative Bacteria**

- The classifications were based not just on morphology, but also on ecological and biochemical properties and, importantly, molecular genetics.

"Volume 1: The Archaea and the Deep-Branching and Phototrophic Bacteria

- **"Volume 1: The Archaea and the Deep-Branching and Phototrophic Bacteria**," published in 1994. This volume is part of the *Bergey's Manual of Systematic Bacteriology*.
- This manual is a comprehensive reference on the classification and identification of bacteria and archaea. In Volume 1, the content focuses on two major groups:

1. **The Archaea**: A domain of single-celled organisms that are distinct from bacteria in terms of their genetic makeup and metabolic pathways. Archaea often thrive in extreme environments (like hot springs or high salinity), and they are classified separately from bacteria.
2. **Deep-Branching Bacteria**: These are bacterial species that represent ancient lineages, which branched off early in the history of life on Earth. These species include the "early" bacteria that are distinct in their genetic traits and often live in extreme or deep-sea environments.
3. **Phototrophic Bacteria**: These bacteria are capable of photosynthesis, using light as an energy source. Phototrophic bacteria include purple sulfur bacteria, green sulfur bacteria, and other groups that are specialized in utilizing light for energy production.

- In terms of classification, Volume 1 was significant in distinguishing between different bacterial and archaeal groups based on molecular, genetic, and morphological characteristics, offering insight into the early divergence of life forms. It also contributed to refining the tree of life based on these distinctions.
- The classification approach used at the time was primarily based on **phenotypic characteristics**, **genetic similarities**, and **phylogenetic relationships**.

Volume 2: The Proteobacteria (Alpha, Beta, Gamma, Delta, and Epsilon Subdivisions)

- "Volume 2: The Proteobacteria (Alpha, Beta, Gamma, Delta, and Epsilon Subdivisions)" from *Bergey's Manual of Systematic Bacteriology* focuses on the classification and detailed descriptions of the **Proteobacteria** group. Proteobacteria are a major group of bacteria, encompassing a vast array of species that are classified into five subdivisions based on genetic, phenotypic, and metabolic characteristics. These subdivisions are:

1. Alpha Proteobacteria:

- This group includes a diverse range of bacteria, many of which are important in environmental, medical, and agricultural contexts.

- They are often associated with symbiotic relationships (e.g., nitrogen-fixing bacteria like Rhizobium that live in the roots of legumes).
- Some notable genera in this subdivision include:
- Rickettsia (responsible for diseases like typhus and Rocky Mountain spotted fever)
- Agrobacterium (used in genetic engineering of plants)
- Brucella (causing brucellosis)

2. Beta Proteobacteria:

- These bacteria often have distinctive metabolic processes, including the ability to oxidize compounds like hydrogen, ammonia, and methane.
- They include a number of medically important pathogens.
- Notable genera include:
- Neisseria (which includes the species Neisseria gonorrhoeae, the causative agent of gonorrhea)

- Burkholderia (responsible for diseases such as cystic fibrosis infections and melioidosis)
- Bordetella (causing whooping cough)

3. Gamma Proteobacteria:

- This is the largest and most diverse subdivision of Proteobacteria, encompassing a wide variety of bacteria.
- Many members are pathogenic or important in industrial applications.

Key genera include:

- Escherichia (including Escherichia coli, a model organism and a common cause of gastrointestinal infections)
- Salmonella (causing food poisoning and typhoid fever)
- Pseudomonas (with species like Pseudomonas aeruginosa, a common opportunistic pathogen)
- Vibrio (including *Vibrio cholerae*, which causes cholera)

4. Delta Proteobacteria:

- These bacteria are typically involved in sulfur or sulfate reduction and play key roles in the sulfur cycle.
- They are often associated with anaerobic environments.
- Examples include:
- Desulfovibrio (sulfate-reducing bacteria)
- Myxococcus (known for its unique fruiting body formation and predatory lifestyle)

5. Epsilon Proteobacteria:

- This group is relatively small but includes some important pathogenic bacteria.
- Many species are found in the gastrointestinal tract of animals, including humans.
- Notable genera include:
- Helicobacter (including Helicobacter pylori, responsible for peptic ulcers and gastric cancer)
- Campylobacter (including Campylobacter jejuni, a leading cause of bacterial foodborne illness)

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Volume 3: The Firmicutes and Tenericutes

Volume 3: The Firmicutes and Tenericutes from Bergey's Manual of Systematic Bacteriology focuses on the **Firmicutes** and **Tenericutes**, two major groups of bacteria that are distinguished by their cellular characteristics and metabolic capabilities.

1. Firmicutes:

The **Firmicutes** are a diverse group of bacteria that are primarily characterized by their thick peptidoglycan cell walls, which make them Gram-positive. This division contains a wide range of bacteria, including important pathogens, fermenters, and industrially useful organisms.

General Features:

- Firmicutes are generally Gram-positive, but there are some exceptions (e.g., *Listeria* is Gram-variable).
- Many are rod-shaped, but cocci forms (like *Staphylococcus* and *Streptococcus*) are also common.
- They are known for their ability to form spores (endospores), which allows them to survive extreme conditions (e.g., *Clostridium* and *Bacillus*).
- Some Firmicutes are obligate anaerobes (e.g., *Clostridium* species), while others can grow in the presence of oxygen (e.g., *Listeria*).

Key Subdivisions/Genera:

- **Bacillales**: This order includes genera such as:
- Bacillus (e.g., Bacillus anthracis, the causative agent of anthrax)
- Clostridium (e.g., Clostridium botulinum, which causes botulism)
- Staphylococcus (e.g., Staphylococcus aureus, which can cause a range of infections)
- Listeria (e.g., Listeria monocytogenes, which can cause listeriosis)
- **Lactobacillales**: This order includes genera important in fermentation and food production:

- Lactobacillus (used in the production of yogurt, sauerkraut, and pickles)
- Streptococcus (e.g., Streptococcus pyogenes, which causes strep throat and other diseases)
- **Mollicutes**: This order includes bacteria that lack a cell wall (e.g., *Mycoplasma*), which is an important feature for distinguishing them from other Firmicutes.

Key Characteristics:

- Many Firmicutes are involved in the breakdown of organic matter in the environment, especially those involved in fermentation processes.
- Firmicutes include both pathogenic species and those used in the production of dairy products, antibiotics, and other industrial products.
- Some Firmicutes form spores that are resistant to heat, radiation, and chemicals (e.g., Bacillus and Clostridium species).

2. Tenericutes:

- The **Tenericutes** represent a small, unique group of bacteria that are characterized by their lack of a cell wall, making them inherently different from most other bacteria.
- Because they lack a rigid cell wall, Tenericutes are highly pleomorphic (can have a variety of shapes) and are more flexible than other bacterial groups. The Tenericutes include the **Mycoplasmas**, which are particularly important in both medical and veterinary microbiology.

General Features:

- Tenericutes are Gram-negative due to the absence of a cell wall.
- They have a simple structure and are generally small in size.
- Their lack of a cell wall makes them resistant to many antibiotics (e.g., penicillin), which target cell wall synthesis.
- They are often parasitic or symbiotic, residing in the tissues of plants, animals, or other microorganisms.
- The cell membrane of Tenericutes often contains sterols, which are not common in other bacteria and help stabilize the membrane.

Key Genera:

Mycoplasma: This is the most well-known genus in the Tenericutes group, and it includes species that are important human and animal pathogens, such as:

- Mycoplasma pneumoniae (causing atypical pneumonia in humans)
- Mycoplasma genitalium (associated with urinary tract infections and other genital infections)

- Mycoplasma bovis (causing pneumonia and mastitis in cattle)

Ureaplasma: Another genus of the Tenericutes, closely related to Mycoplasma, that includes species causing urinary tract infections and other genital infections in humans.

Key Characteristics:

- Because Tenericutes lack a cell wall, they can pass through filters that would normally retain other bacteria.
- They are highly adapted to parasitic life, often living in or on host cells, relying on host resources for survival.
- Their ability to evade immune detection and their lack of a cell wall make them particularly difficult to treat with conventional antibiotics.

Volume 4: The Actinobacteria and other Gram-Positive Bacteria

- **Volume 4: The Actinobacteria and Other Gram-Positive Bacteria** from Bergey's Manual of Systematic Bacteriology focuses on the **Actinobacteria** and other Gram-positive bacterial groups that have distinct characteristics in terms of morphology, metabolism, and ecological roles.
- This volume explores the diversity of Gram-positive bacteria, including those that are important for human health, industrial applications, and environmental processes.

1. Actinobacteria:

- The **Actinobacteria** are a large and diverse group of bacteria, many of which are Gram-positive, with a high GC (guanine-cytosine) content in their DNA. They are found in a variety of habitats, from soil and water to the human body. This group includes some of the most well-known and medically important bacteria.

General Features:

- High GC content in their DNA (above 55%).
- Often have complex cell wall structures, which can include mycolic acids (long-chain fatty acids) in some species, contributing to a waxy coat that makes them resistant to desiccation and certain chemicals.
- Many are obligate aerobes, although some are facultative anaerobes or obligate anaerobes.
- Actinobacteria often exhibit a filamentous morphology or can form branching structures resembling fungi (e.g., Actinomyces and Streptomyces).
- Several species are known for their ability to produce secondary metabolites, including antibiotics and other bioactive compounds.

Key Genera:

- **Streptomyces**: This genus is famous for its production of a wide range of antibiotics,

including streptomycin, tetracycline, and erythromycin. *Streptomyces* species are abundant in soil and are a major source of natural products used in medicine.

- **Mycobacterium**: This genus includes important human pathogens like Mycobacterium tuberculosis (causing tuberculosis) and Mycobacterium leprae (causing leprosy). These bacteria have a characteristic cell wall rich in mycolic acids, which makes them resistant to staining and contributes to their pathogenicity.
- **Corynebacterium**: This genus includes Corynebacterium diphtheriae (the causative agent of diphtheria). Many species are found in the human microbiota, but some are pathogens.
- **Nocardia**: Nocardia species are soil-dwelling organisms that can cause infections, particularly in immunocompromised individuals. They are known for their ability to degrade hydrocarbons.
- **Actinomyces**: These bacteria are part of the normal microbiota in the human mouth and gastrointestinal tract, but they can also cause actinomycosis, a chronic infection characterized by abscess formation.
- **Bifidobacterium**: These bacteria are commonly found in the intestines of humans and other animals. They are important for gut health and are used as probiotics in foods like yogurt.

Significance:

- **Antibiotic production**: Streptomyces and related genera are major sources of antibiotics, which have been pivotal in treating a range of bacterial infections.
- **Pathogenicity**: Many Actinobacteria are important pathogens, such as Mycobacterium tuberculosis and Corynebacterium diphtheriae. These bacteria have unique mechanisms of survival and virulence.
- **Industrial and Environmental Roles**: Many species are involved in the breakdown of organic matter, including plant material, and some have applications in biotechnology (e.g., the production of biofuels or bioremediation).

2. Other Gram-Positive Bacteria:

- Apart from the Actinobacteria, Volume 4 also covers other **Gram-positive bacteria** that do not belong to the Actinobacteria but are still characterized by a thick peptidoglycan cell wall. These include several well-known groups and pathogens.

Key Genera:

- **Firmicutes**: This is a group of Gram-positive bacteria, discussed in Volume 3, which includes important genera such as Bacillus, Clostridium, Staphylococcus, and Lactobacillus. These bacteria are found in a variety of environments, ranging from the human body (e.g., Staphylococcus aureus) to soil (e.g., Bacillus species).

- **Lactobacillales**: This order includes bacteria that are important in fermentation, including Lactobacillus (used in yogurt production) and Streptococcus (some species cause strep throat, while others are used in dairy fermentation).
- **Enterococcus**: These are Gram-positive cocci found in the intestines of humans and other animals. Some species, like Enterococcus faecalis, are important in human infections, particularly in urinary tract and endocardial infections.
- **Listeria**: Listeria monocytogenes is a pathogen that can cause listeriosis, a foodborne illness, and can cross the blood-brain barrier, leading to meningitis in vulnerable individuals.

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- **Rhodococcus**: These bacteria are found in soil and can degrade a variety of organic compounds, including hydrocarbons. Some species are pathogenic to animals.

3. Key Characteristics of Gram-Positive Bacteria in Volume 4:

- **Cell Wall Structure**: The majority of Gram-positive bacteria, including Actinobacteria, have a thick peptidoglycan layer, which gives them their Gram-positive staining. However, some, like Mycobacterium, also have mycolic acids in their cell walls, providing additional resistance to environmental stressors.
- **Metabolic Diversity**: Gram-positive bacteria exhibit a broad range of metabolic abilities. Some are obligate aerobes, others facultative anaerobes, and still others obligate anaerobes. Some, like the lactic acid bacteria (e.g., Lactobacillus), are fermentative.
- **Pathogenicity and Antibiotic Resistance**: Many Gram-positive bacteria are important human pathogens. For example, Staphylococcus aureus can cause skin infections, pneumonia, and sepsis, while Clostridium botulinum can cause botulism. Antibiotic resistance in Gram-positive bacteria (e.g., Methicillin-resistant Staphylococcus aureus or MRSA) is a major concern in clinical settings.

4. Ecological and Industrial Roles:

- **Degradation of Organic Matter**: Many Actinobacteria and other Gram-positive bacteria play crucial roles in nutrient cycling, particularly the degradation of complex organic compounds like cellulose and lignin.
- **Antibiotics and Natural Products**: Actinobacteria, especially Streptomyces, are a major source of antibiotics. The discovery of antibiotics like streptomycin and tetracycline revolutionized medicine in the 20th century.
- **Probiotics and Food Production**: Some Gram-positive bacteria, particularly from the genera Lactobacillus and Bifidobacterium, are used in the production of fermented foods and as probiotics for gut health.

5. Significance in Medicine:

- **Pathogenic Gram-Positive Bacteria**: As mentioned earlier, several Gram-positive

bacteria are significant human pathogens. The Streptococcus, Staphylococcus, and Clostridium genera, among others, are responsible for a variety of infections ranging from mild to life-threatening.

- **Antibiotic Resistance**: The emergence of antibiotic-resistant strains, particularly in the Staphylococcus and Enterococcus genera, poses a significant challenge to public health. The spread of resistance genes is a key focus of modern bacteriology.

Volume 5: The Bacteroidetes, Planctomycetes, and other Gram-Negative Bacteria

- **Volume 5: The Bacteroidetes, Planctomycetes, and Other Gram-Negative Bacteria** from Bergey's Manual of Systematic Bacteriology focuses on a diverse set of **Gram-negative bacteria** that are not only biologically distinct but also occupy important ecological niches.
- This volume explores the taxonomy, physiology, and significance of various Gram-negative bacterial groups, particularly those belonging to the **Bacteroidetes**, **Planctomycetes**, and other significant, but sometimes less well-known, bacterial divisions.

1. Bacteroidetes:

- The **Bacteroidetes** are a diverse phylum of Gram-negative bacteria that are commonly found in soil, the human gastrointestinal tract, and the environment. These bacteria play significant roles in the degradation of complex organic materials, especially polysaccharides. **(PG TRB 2020-2021)**

General Features:

- Bacteroidetes are typically **rod-shaped** or **spiral** bacteria with a Gram-negative cell wall structure (outer membrane and thin peptidoglycan layer).
- Many species are **anaerobic** or **facultative anaerobes**, and they often engage in the breakdown of complex carbohydrates, such as polysaccharides, in the intestines.
- Some species are part of the normal microbiota of humans and animals, especially in the intestines, where they contribute to digestion.

Key Genera:

- **Bacteroides**: The genus *Bacteroides* is one of the most prominent in the human gut microbiota. These bacteria are involved in the fermentation of polysaccharides, contributing to nutrient absorption. However, Bacteroides species can also be opportunistic pathogens in certain infections (e.g., abdominal infections, septicemia).
- **Prevotella**: Closely related to Bacteroides, Prevotella species are abundant in the human mouth and gastrointestinal tract. Some species can cause infections in humans, including periodontal disease.

- **Flavobacterium**: Found in aquatic environments, Flavobacterium species are important in nutrient cycling, especially in the breakdown of organic material in water.

Significance:

- **Gut Health**: *Bacteroides* and related genera are crucial for the digestion of complex carbohydrates, helping humans and other animals process dietary fibers.
- **Pathogenic Potential**: While many species of Bacteroidetes are part of the normal flora, some can become pathogenic under certain conditions, particularly in polymicrobial infections in deep tissues (e.g., abscesses, peritonitis).
- **Environmental Degradation**: Some species are involved in the degradation of complex organic compounds in the environment, playing a role in carbon and nitrogen cycling.

2. Planctomycetes:

Planctomycetes is a unique phylum of Gram-negative bacteria with distinctive features, including a **double membrane** surrounding their cells, giving them an appearance similar to **eukaryotic cells**.

General Features:

- These bacteria have a **distinctive cell structure**, including a double membrane around the cell, often described as an **intracellular compartment**.
- **Planctomycetes** lack peptidoglycan in their cell walls, which is unusual for Gram-negative bacteria.
- Many species are **aquatic** and have been found in environments such as fresh water, seawater, and in wastewater treatment systems.
- Some species are **anaerobic** or **facultative anaerobes** and participate in nitrogen cycling, particularly in **anammox reactions** (anaerobic ammonium oxidation).

Key Genera:

- **Planctomyces**: This genus contains species that are typically found in aquatic environments and are notable for their unique cellular structure and ability to participate in the anammox process.
- **Anammoxobacter**: Known for their ability to perform **anaerobic ammonium oxidation**, these bacteria are important in the nitrogen cycle and are often found in environments with low oxygen concentrations.
- **Pirellula**: A genus of **marine** bacteria that has a distinct morphology and is involved in **biogeochemical cycles** in aquatic systems.

Significance:

- **Nitrogen Cycling**: Planctomycetes, especially the genera involved in the anammox

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BOTANY

UNIT-IX (Volume 2)

Microbiology and Plant Pathology, Bacteria, Virology, Plant Pathology

Your Success is Our Goal...

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MICROBIOLOGY AND PLANT PATHOLOGY

PG TRB (2025-2026)

UNIT – 9 (VOL-2)

FIRST EDITION

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Syllabus

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Virology:

General characteristics, Classification of plant viruses (ICTV,1970), structure and multiplication of plant viruses. Bacteriophage: Structural characteristics and multiplication. Virion, viroid, virusoids and prions. Isolation and purification of plant viruses.

Plant Pathology:

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8. Plant Pathology (Pathogen and Plant Disease) - By Pandey B.P.

9. Plant Pathology - By Dr. P.D. Sharma

10. Microbiology & Plant Pathology - By Dr. P.D. Sharma

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UNIT IX (VOL 2) - MICROBIOLOGY AND PLANT

2. VIROLOGY

INTRODUCTION

- Viruses may be generalized to define as 'very small sized etiological agents of disease that are capable of passing through filters that retain even bacteria, increase only in the presence of living cells, and give rise to new strains by mutation'.
- Mayer (1886) showed that the juice from the infected plants of tobacco could reproduce the disease if applied to healthy plants. The Russian botanist Dimitri Ivanowski (1892), demonstrated that the causal organism of tobacco mosaic could even pass through the finest porcelain filter that withholds bacteria. Ivanowski also showed that this filterate was capable of transmitting the disease to healthy susceptible plants.
- He also indicated that these causal organisms were even smaller than bacteria. Beijerinck (1898) a Dutch microbiologist, showed that the causal agent or tobacco mosaic could diffuse through an agar membrane and was therefore liquid in nature such a liquid causal agent of tobacco mosaic was called by Beijerinck as "Contagium vivum fluidium' or 'living infection fluid'.
- Bacteriophages (viruses that parasitise bacteriA) were discovered by the French scientist D. Herelle (1917) who found that some agent was destroying his cultures of bacilli.
- Schelsinger (1933) was the first to determine the decomposition of virus. He showed that a bacteriophage consists of only protein and DNA. Bowden (1964) defined viruses as 'submicroscopic, infective entities that multiply only intracellularly and are potentially pathogenic. According to Hahon (1964) viruses are 'bits of infectious heredity in search of a chromosome'.

- Some define viruses as 'infectious nucleoproteins'. The word virus is derived from the latin language meaning 'poisonous liquid or 'poison'. In 1935 Stanley crystallized the virus causing tobacco mosaic disease, and demonstrated that the crystals retained their infectivity when inoculated into healthy plants Hershey and Chase (1952) studied the T2 bacteriophage and demonstrated that The genetic information is carried in the phage DNA and (2) that infection is the result of penetration of viral DNA into cells.
- The nucleic acid fraction of the virus is the actual infectious agent was first shown by Gierrer and Schramm (1956). Phycophages were first isolated by Schafferman and Morris (1963) from blue green alga LyngbtJa. The phage isolated by them was found to infect Plectono1lema and Phormidiunl also (hence named LPP-1), these are cyanophages. Mycophages were first discovered in mushroom (Agaricus bisp0nls) by Sinden in 1957.

2.1 GENERAL CHARACTERS OF VIRUSES

(1) They do not occur free in nature but act as obiigate intracellular parasite.

(2) They are extreme microscopic structure which can only be seen by electron microscope.

(3) Mainly the size ranges from 100-2000 millimicron.

(4) They can not be filtered by bacterial filters.

(5) The genetic material is either DNA or RNA which occurs in the form of single molecule and can be single or double stranded.

(6) A single virus particle is known as virion which lacks functional autonomy.

(7) They lack their own enzyme system but interact with the host enzyme system and synthesize new virus particles. Thus, they have a. master and slave relationship.

(8) Outer capsid of virus is proteinaceous and harmless and provide cellular specificity to the virus.

(9) They are intracellular obligate parasite and can't be cultured on artificial culture media.

(10) All animal and plant viruses have a narrow host range while others show a broad host range.

(11) They show replication.

(12) They are highly infectious and spread disease very quickly.

(13) They show special kind of pathogenecity i.e. they cause disease at particular temperature. Most of virus become inert at 56-69°C (for 30 minutes)

(14) They are haploid.

(15) They are uneffected by antibiotics.

(16) They show life between 5-9 pH.

(17) They remain active for a long time when kept in 50% glycerol solution.

(18) The extract of virus become inert at high pressure and high sound frequency.

(19) They get precipitated with ethyl alcohol and acetone.

(20) They can be inerted by treatment with ultraviolet rays, pyridine, urea and hydrogen peroxide.

(21) They can be crystallized.

(22) They show response toward temperature, radiation and chemical substances.

(23) They lack cell wall, nucleus, protoplasm and cell organelles.

How do Virus differ from Bacteria and Mycoplasmas?

Viruses differ from bacteria and mycoplasmas in:

i) not possessing any cellular organization.

(ii) Not growing on inanimate media.

(iii) not multiply by binary fission.

(iv) Not possessing both DNA and RNA together.

(v) Not possessing ribosome.

(vi) Not showing any sensitivity to antibiotics.

(vii) Showing sensitivity to interferon.

2.1.1 NATURE OF VIRUSES

- The nature of viruses is still not clear, because it is not easy to define them within the accepted framework of living or non living organisms. Some virologist regard viruses as animate object (when present inside the host cell) whereas other consider them inanimate (when present outside the host cell).

Viruses are living because:

(i) They show growth and multiplication (only inside the host cell).

(ii) They have genetic material i.e. DNA/RNA.

(iii) They can direct protein synthesis (though they use host machinery for it).

(iv) They show mutation.

(v) They can be transmitted from the diseased host to the healthy ones or posses the ability to infect.

(vi) They react to heat, chemicals and radiation and also shows irritability, a character of only living organisms.

(vii) They posse's genetic continuity and have definite races/strains.

(viii) Similarity between nucleoproteins of viruses with the protein and nucleic acid of living organisms.

Viruses are non-living because:

(i) They can be crystallized (Stanley, 1935)

(ii) They behave as inert chemicals outside the host cell.

(iii) A cell wall or cell membrane of any type is absent in viruses.

(iv) They do not show functional autonomy.

(v) They do not respire or excrete or they do not show any sign of metabolism except reproduction.

(vi) They lack any energy producing enzyme system.

- Therefore, the contention that 'viruses are viruses' (Lwoff *et al.,* 1966) and nothing else 'stands on the top'. According to regressive theory of evolution put forward by Lwoff, some primitive microorganism (like bacteriA) become endoparasitic on some host and gradually lost synthetic enzymes to become today's viruses.
- viruses are super parasities. It will thus be seen that viruses do not show all the characteristic of typical living organisms. They however possess two fundamental characteristic of living systems.
- Firstly, they contain nucleic acid as their genetic material. The nucleic acid contains instruction for the structure and function of the virus. Secondly, they can reproduce themselves, even if only by using the host cell's synthesis machinery. Because of such characters, some virologist considers viruses as a transition stage between living and non living world. They are living organism with some non living characters.

2.1.2 OCCURRENCE

- The occurrence of viruses in the cells of bacteria and higher plants and animals is well established.
- **Plant viruses:** Most plant viruses have been found in angiosperm (flowering plants). Relatively few viruses are known in gymnosperm, ferns, fungi or algae. Plant viruses are of great economic importance, since they cause plant diseases in a variety of crops.
- **Animal viruses**: Virus diseases are known in a variety of vertebrates including fish, amphibian, birds and mammals. Important virus diseases of humans include poliomyelitis, small pox, rabies, mumps, measles, yellow fever, influenza and encephalitis.
- **Bacteriophages:** Viruses have been found in practically all groups of bacteria. The host range is confined within bacterial groups. A bacteriophage may multiply only in certain strains of E. coli.

2.1.3 VIRION:

- A single infective particle of virus is called as virion. It consists of nucleic acid core surrounded by a protein coat or capsid. **(PG TRB 2001)** The capsid with enclosed nucleic acid is called nucleocapsid.

- **Viroids:** These are the smallest infectious agents causing diseases in host. They consist solely of a protein free low molecular weight (75,000 - 1,25,000 dalton) with 243-360 nucleotides and small fragments of double stranded RNA molecules. They are also known as naked virus, meta virus or pathogene.
- Evidence shows that viroids replicate by direct RNA copying in which all components required for viroid replication including RNA polymerase are provided by the host. During viroid replication, the circular (+) strand of the viroid is replicated while it acts as a rolling drum producing multimeric linear strands of (-) RNA.
- The linear (-) strand then serves as a template for replication of multimeric strand (+) of RNA. The (+) RNA is subsequently processed (cleaved) by enzymes that release linear, unit length viroid (+) RNA's which circularize and produce many copies of the original viroid RNA.
- Viroids apparently interfere with the host metabolism in ways resembling those of viruses but of what way is still unclear. It has been shown that both virus specific RNA's synthesized during infection and viroid RNA *in vitro* activate a protien kinase enzyme, which in turn activates other cellular enzymes while it impedes the initiation of protein synthesis.
- As viroid strain that cause mild to severe plant symptoms activate the protein kinase more than 10 times as much as mild strains, it is possible that activation of the protein kinase represents the triggering event in viroid pathogenesis and in disease development by the plant.
- Viroids are spreaded from diseased to healthy plants primarily by mechanical means, i.e. sap carried through hands or tools during cultural practices while some are transmitted by pollen and seed.
- Viroids survive in nature outside the host or in dead plant matter for periods of time from few minutes to few months. They are quiet resistant to high temperature and can't be inactivated in infected plant by Heat treatment.
- The control of diseases caused by viroids is based on the use of viroid-free propagating stock, removal and destruction of viroid infected plants and washing of hands or sterlizing of tools after handling viroid infected plants before moving on to healthy plants.
- Potato spindle tuber viroid (PSTvd) is the first recognized viroid, which consist of 359 nucleotides under E.M. Purified PSTvd appears as short strands about 40 nm long and has the thickness of a dsDNA.
- Viroids seem to be associated with cell nuclei particularly the chromatin and possibly with the endomembrane system of the cell. It was first discovered by Diener (1971) as the causal agent of potato spindle tuber disease.

- Other viroid caused disease reported so far are- tomato bunchy top disease, chrysanthemum stunt disease, coconut cadang disease, tomato apical stunt disease, Avocado sunblotch etc.

They undergo replication by using the host enzyme system. Their transmission is through the cromatin material of host cell.

2.1.4 VIRUSOIDES:

- It has been introduced by Rendle *et al* (1981). "Virusoides are the viroides which require RNA of the supportive virus for replication".
- At present it has been reported only in Australia with few examples of its host. viz. one virusoide is reported along with velvet tobacco mosaic virus. Other virusoides are attached as satellite along with other virus RNA.
- These virusoides undergo replication with the help of RNA of the helper virus inside the host cell.

2.1.5 PRIONS:

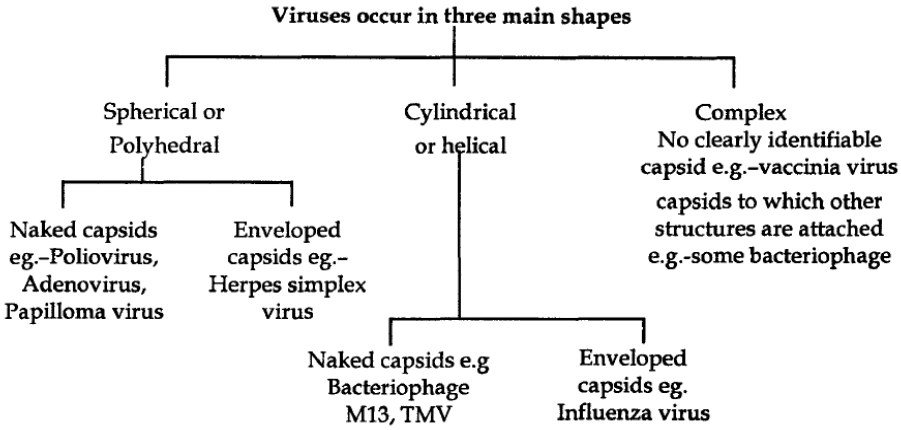
- Haig and Claske (1966) discovered a subviral infectious agent which was later called as prions by Prusiner *et al.* Later Prof. Prusiner has been awarded nobel Prize (1997) for medicine for the discovery of prions.
- These prions are the causal agent of scrapie disease (a degenerative disorder of central nervous system) of sheep and goat. These prions have no nucleic acid *(DNAj* RNA) but they are made up of only 2-3 molecules of protein only.
- Prions are 100 times shorter than viruses and are heterogenous in nature. A single prion rod is made up of about 1000 prion molecules. It is 100-200 nm long and 10- 20 nm in diameter. Other disease caused by prions are Parkinson's disease, multiple sclerosis, Gerstmann Stransslar syndrome and Creutzfeldt-Jakob disease.

2.1.6 SIZE AND STRUCTURE OF VIRUSES

- The size of viruses is variable. Most viruses are much smaller than bacteria. Their size ranges from 10 nm - 250 nm.
- The size of viruses is determined by electron microscopy, ultra centrifugation and by filtration through colloid ion membrane of known pore diameter.
- The smallest virus is coliphage F2 measuring about 2 nm. The smallest plant virus is satellite tobacco necrosis virus measuring 17nm. The longest known plant virus is citrus tristeza virus-rod shaped measuring 2000 x 12 nm.
- Foot and mouth virus of cattle is the smallest animal virus measuring about 10 nm.
- Pox viruses are the largest and most complex animal viruses. Parrot fever virus measuring 400 nm.

Structure of Viruses

- The intact virus unit or infectious particle is called the virion. Each virion consists of a nucleic acid core surrounded by a protein coat (capsid) to form the nucleocapsid. The nucleocapsid may be naked or may be surrounded by a loose membranous envelope.
- It is composed of a number of subunits called capsomeres. The capsid protects the nucleic acid core against the action of nucleases. Structurally viruses occur in three main shapes viz. spherical or polyhedral, cylindrical or helical and the complex type.



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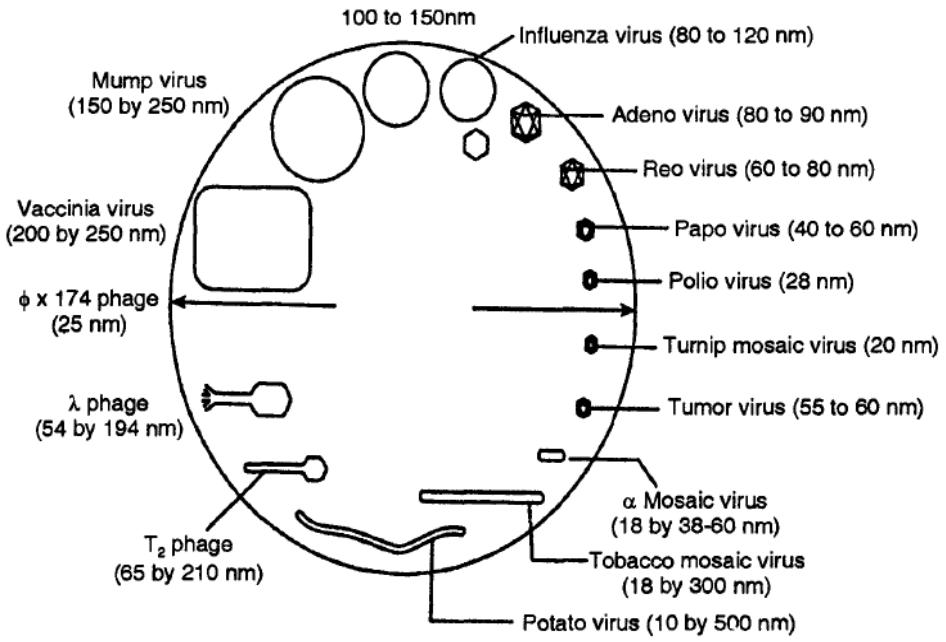
(I) POLYHEDRAL (ICOSAHEDRAL) SYMMETRY

- Crick & Watson have shown that the polyhedral capsids can nave three possible types of symmetry viz. Tetrahedral, octahedral and icosahedral. Icosahedral is the most efficient shape for the packing and bonding of subunitsof a near spherical virus. In icosahedral symmetry a large number of intermolecular
- bonds can be formed in this type of structure and is therefore has low free energy. An icosahedron is a regular polyhedron with 20 faces formed by equilateral triangles and 12 intersecting points or corners.
- Each capsid consist of many capsomeres. Each capsomere is composed of a few monomers which form polygonal rings, each with a central space of up to 40 AO. The monomers are the structural units and are made up of one or more polypeptide chains. There are two types of capsomeres:
- (i) Pentamers or pentagonal capsomere is made up of 5 monomers.
- (ii) Hexamers or hexagonal capsomere consist of 6 monomers.
- The monomers are held tog~ther by bonds, each monomers having bonds with two neighbouring monomers. The capsomeres are also held together by bonds. These bonds are weaker than the bonds between the monomers. The minimum number of capsomeres can theoretically be 12 followed by 32, 72, 92, 162. of these capsomeres 12 are pentamers occupying the 12 corners, while the rest are hexamers.



Size and shape of various viruses

(II) HELICAL SYMMETRY

- The helical capsid consists of monomers arranged in a helix around a single rotational axis. The monomers curve into a helix because they are thicker at one end than the other. Helical capsids may be naked (e.g. the tobacco mosaic virus) or surrounded by an envelope (e.g. influenza virus).

Tobacco Mosaic Virus

- Virus is rod shaped about 300 nm x 15-18 nm in diameter. X-ray diffraction studies have shown that the virus consists of a protein tube with a lumen of 20 A 0 enclosing a single strand of helically coiled RNA.
- The tube is made up of a number of identical sub units (monomers) of proteins arranged in a helical manner. Studies by Franklin and her co-workers have shown that there are 49 subunits of protein for three turns of the helix, thus giving a total of 2,130 subunits for the rod.
- Each subunit has a molecular weight of 17, 500 and consist of single poly chain made up of 158 amino acids. The RNA is a single stranded moleculer coiled into a helix 80Ao in diameter. It follows the pitch of the protein helix. Each tum of RNA helix contains about 49 nucleotides with a pitch of 23°.

(III) COMPLEX SYMMETRY

Complex viruses are divided into two groups:

(a) Those without identifiable capsids.

(b) Those with capsids to which are attached additional structures. Vaccinia virus is an example of a virus without a definite capsid. The nucleic acid is surrounded by several coats.

- Bacteriophages are the virus that infect bacteria, was discovered independently by Frederick. Twort in England 1915 and by Felix d' Herelle at the Pasteur Institute in Paris in 1917. D'Herelle noted that something was dissolving or lysing their bacterial cultures of Staphylococci and this lytic effect could be transmitted from colony to colony.
- Even high dilution of material from a lysed colony that had been passed through a bacterial filter could transmit the lytic effect. However, heating the filterate destroyed its lytic properties. And this lytic phenomenon is commonly called as Twort-d'Herelle phenomenon.
- In nature 'Phages' occur commonly in close association with bacteria. They play an important role in the transmission of genetic information between bacteria by transduction process.

- Bacteriophages provide the only convenient model to study the virus-host interaction at cellular and molecular levels. All most every group of bacteria is attacked by one or other phage. But E. coli has been studied most extensively from this point of view. Bacteriophage attacking E. coli are called coliphages and are designated T type.
- These were numbered Tl, T2, T3Tl7 by Max Delbruck (1938). The best known and most thoroughly studied are T2, T4, T6 which are collectively called T -even phages. T 3' T s are called T -odd phages.
- There are two main types of bacterial viruses, lytic or virulent and temperate or avirulent. When lytic phages infect cells, the cells respond by producing large number of new viruses.
- That is at the end of the incubation period the host cell bursts or lyses, releasing new phages to infect other host cells. This is called a lytic cycle. In the temperate type of infection, the result is not so readily apparent. The viral nucleic acid is carried and replicated in the host bacterial cells from one generation to another without any cell lysis.

Bacterial viruses may be grouped into six morphological types:

- A = This is most complex type has a hexagonal head, a rigid tail with a contractile sheath and tail fibers. e.g. $T_{2'}$ $T_{4'}$ T_6 (T -even).
- B = Similar to A, this type has a hexagonal head. However it lacks a contractile sheath, its tail is flexible and it mayor may not have tail fibers. ego Coliphages like T_l and T_5.
- C = This type is characterized by a hexagonal head and a tail shorter than the head. The tail has no contractile sheath and mayor may not have tail fibers. Eg. Coliphage T_3 and T_7
- D = This type has a head made up of large capsomeres, but has no tail eg.Coliphages, ϕ x 174, S_{13},

- E = This type of head made up of small capsomeres but has no tail ego Coliphages F_2, MS_2.
- F = This type is filamentous. ego Coliphages Fd, FI.
- G = Pleomorphic, no detectable capsid, envelope contain lipid ego MV - L2.
- Type A,B,C show morphology unique to bacteriophages. The morphological types in groups D and E are found in plant and animal (including insect) virus. The filamentous form of group F is found in some plant viruses.
- Bacteriophages of the T-even Series (T2' T4, T6) It is an example of complex viruses with capsids and attached structures. T4 bacteriophage is tadpole shaped, with head and tail regions. Head capsid is 95 x 65 nm and has the form of a prolate icosahedron. It is made up of about 2,000 similar subunits and is packed with circular double stranded DNA (500 nm long).
- Head capsid consist of two 10-faceted equatorial bands with a pyramidal vertex at either end. The tail has helical symmetry. Thus, the bacteriophage shows a combination of icosahedral symmetry and helical symmetry (binal symmetry). The tail consist of a core tube 80Ao in diameter, through which DNA passes out surrounded by a protein tail sheath.
- The sheath consists of 144 subunits arranged in 24 rings of 6 subunits each. The sheath is connected to a thin disc, called the collar at the upper end and a base plate at the lower end.
- The base plate is hexagonal and has a pen or spike at each corner. From each of the six corners is also given off a long, thin tail fibre. $1300A^0$ long, which serves for the attachment of the bacteriophage to the host cell.

MULTIPLE CHOOSE QUESTION-19

1. Which of the following is a characteristic feature of viruses?

A) They can reproduce independently
B) They are composed of a single type of cell
C) They are obligate intracellular parasites
D) They are capable of photosynthesis

2. Viruses are composed of which of the following?

A) Nucleic acid (DNA or RNA) and a protein coat
B) Cell membrane and organelles
C) Chlorophyll and protein
D) Nucleic acids and lipids

3. Which of the following is true about the replication of viruses?

A) They replicate through binary fission

B) They require a host cell to replicate
C) They can replicate on their own outside of a host
D) They replicate by budding from a host cell

4. What is the protein coat of a virus called?

A) Capsid B) Envelope C) Peptidoglycan D) Nucleosome

5. Viruses can infect which of the following?

A) Only plant cells
B) Only animal cells
C) Only bacteria
D) Bacteria, plants, and animals

6. Which of the following statements is true about viral genomes?

A) All viruses have RNA as their genetic material
B) Viral genomes can be either DNA or RNA, but not both
C) Viral genomes are typically large and complex
D) Viruses lack any genetic material

7. What type of nucleic acid is found in retroviruses?

A) Double-stranded DNA
B) Single-stranded RNA
C) Double-stranded RNA
D) Single-stranded DNA

8. Which of the following is NOT a characteristic of viruses?

A) Viruses lack cellular structures
B) Viruses can only replicate inside host cells
C) Viruses have a complex structure with organelles
D) Viruses can cause diseases in humans, animals, and plants

9. What is the outermost layer of some viruses, made up of lipids and proteins, called?

A) Capsid
B) Envelope
C) Glycoprotein
D) Peptidoglycan

10. Which of the following is a type of virus that infects bacteria?

A) Bacteriophage
B) Retrovirus
C) Adenovirus
D) Influenza virus

2.2 CLASSIFICATION OF VIRUS ICTV (1970)

- The International Committee on Taxonomy of Viruses (ICTV) is a committee which authorizes and organizes the taxonomic classification of viruses.
- They have developed a universal taxonomic scheme for viruses and aim to describe all the viruses of living organisms. Members of the committee are considered to be world experts on viruses.

- The committee formed from and is governed by the Virology Division of the International Union of Microbiological Societies. Detailed work such as delimiting the boundaries of species within a family is typically done by study groups, which consist of experts in the families. The committee also operates an authoritative database (ICTVdB) containing taxonomic information for 1,950 virus species, as of 2005. It is open to the public and is searchable by several different means.

The official objectives of the ICTV are: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1. To develop an internationally agreed upon taxonomy for viruses.
2. To develop internationally agreed upon names for virus taxa, including species and subviral agents.
3. To communicate taxonomic decisions to all users of virus names, in particular the international community of virologists, by publications and via the Internet.
4. To maintain an index of virus names.
5. To maintain an ICTV database on the Internet, that records the data that characterize each named viral taxon, together with the common names of each taxon in all major languages.

- Proposals for new names, name changes, and the establishment and taxonomic placement of taxa are handled by the Executive Committee of the ICTV in the form of proposals.
- All relevant ICTV subcommittees and study groups are consulted prior to a decision being made. The name of a taxon has no status until it has been approved by ICTV, and names will only be accepted if they are linked to approved hierarchical taxa.
- If no suitable name is proposed for a taxon, the taxon may be approved and the name be left undecided until the adoption of an acceptable international name, when one is proposed to and accepted by ICTV.
- Names must not convey a meaning for the taxon which would seem to either exclude viruses which are rightfully members of that taxa, exclude members which might one day belong to that taxa, or include viruses which are members of different taxa.
- In 1970, the International Committee on Taxonomy of Viruses (ICTV) was in the early stages of standardizing the classification system for viruses. The system was not as refined or detailed as it is today, and it was based on a variety of physical, chemical, and biological characteristics.
- The primary aim was to group viruses according to shared features to facilitate better understanding, research, and communication about them. Below is a detailed study of how viruses were classified by the ICTV in 1970.

1. Nucleic Acid Type

- One of the most fundamental criteria for virus classification in 1970 was the type of nucleic acid they contained. This was categorized into two major groups: DNA viruses and RNA viruses.
- **DNA Viruses**: Viruses whose genetic material was composed of deoxyribonucleic acid (DNA). This group was further divided into:
- **Double-stranded DNA (dsDNA)** viruses: Viruses with two complementary strands of DNA.
- **Single-stranded DNA (ssDNA)** viruses: Viruses with a single strand of DNA.
- **RNA Viruses**: Viruses whose genetic material was ribonucleic acid (RNA). They were also subdivided based on whether the RNA was single-stranded or double-stranded, and how it was organized:
- **Single-stranded RNA (ssRNA)** viruses: Viruses with RNA as a single strand.
- **Double-stranded RNA (dsRNA)** viruses: Viruses with two strands of RNA.
- **Positive-sense (+ssRNA)** and **Negative-sense (-ssRNA)**: Some RNA viruses were distinguished based on whether their RNA was directly translated into protein (positive-sense) or required transcription to a complementary strand before translation (negative-sense).

2. Capsid Symmetry

- The physical shape of the virus was another important factor for classification.
- The virus capsid, which is the protein shell surrounding the viral genome, could exhibit different symmetrical structures:
- **Icosahedral Symmetry**: Many viruses were categorized as having icosahedral symmetry, which is a symmetrical, spherical shape formed by 20 equilateral triangular faces. Examples include the **Picornaviridae** family.
- **Helical Symmetry**: Some viruses were classified as having a helical capsid, which is a rod-like or spiral structure.
- In these viruses, the protein subunits form a helical structure around the genetic material. The **Tobacco Mosaic Virus (TMV)** is a classic example.
- **Complex Symmetry**: Some viruses have irregular or complex capsids that do not fit into the simple categories of icosahedral or helical shapes.
- These viruses were typically larger and more structurally elaborate. For example, **Bacteriophages** (viruses that infect bacteriA) exhibit complex symmetry.

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Unit X

INTRODUCTION TO PLANT TISSUE CULTURE

SYLLABUS

Basics of plant tissue culture – concepts in plant tissue culture. Techniques. Micropropagation, organ culture, meristem culture, protoplast culture and haploid plant production. Callus induction, Cell suspension culture, somatic embryogenesis, synthetic seed technology. Conservation of Plant genetic resources. Application of cell culture systems in metabolic engineering. Concepts and application of nanobiotechnology: Application of nano particles in Agriculture, environment and medicine. Impact of nano-science and nano-biotechnology to society.

Genetic Engineering:

Principles of rDNA technology. Molecular tools in Genetic engineering. Cloning vector – Plasmids – types, Mechanism of plasmids, Isolation of plasmids. Cosmids and phage vectors. Construction of Genomic library, polymerase chain reaction (PCR), Molecular Markers (RAPD, RELP and AFLP). Blotting techniques (Southern, Northern and Western blots). Sequencing methods for DNA. Genetic transformation and development of transgenic plant for insect, herbicide and viral resistances, Golden rice, Edible vaccines, Bio-farming, bioremediation and bioprospecting, salt and drought tolerant plants, enhancement of shelf life of flowers and fruits. Socio-economic and ethical aspects of biotechnology. Environmental laws; Intellectual property rights; World Intellectual Property Organization (WIPO) GATT, TRIPS, PBR and Farmers rights and its role. Ecological impact and biosafety issues of GM crops.

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5.Dubey. R.C.A - Text Book of Biotechnology..

6. Brown T.A - Genomes.

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9.T.A. BROWN - Gene Cloning And DNA Analysis

MORE REFERENCE

- PGTRB Previous Year Question Papers
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UNIT - X

TCA

1. INTRODUCTION TO PLANT TISSUE CULTURE

- Plant tissue culture is a significant contribution in micropropagation of ornamental and forest trees, production of pharmaceutically interesting compounds and plant breeding for improved nutritional value of staple crop plants as well as in the improvement of tree species.
- Plant tissue culture can provide high-quality planting material for the fruits, vegetables and ornamental plants and forest tree species throughout the year, irrespective of season and weather, thus opening new opportunities to producers, farmers and nursery owners.
- The biotechnological approaches like haploid induction, somaclonal variation, etc. to improve traits are also its important applications.
- Plant tissue culture is a noble approach to be used in bioproduction, bioconversion or biotransformation of the valuable secondary products for large-scale production and biosynthetic studies.
- Plant tissue culture has been routinely done for conservation of germplasm to be used for improving secondary metabolite production and agronomic traits of crops to increase yields. **CSIR NET JUNE 2012)**
- Production of artificial seeds has unravelled new vistas in in vitro plant biotechnology, such as large-scale clonal propagation, delivery of clonal plantlets, germplasm conservation and breeding of plants in which propagation through normal seeds is not possible.

1.1 BASICS OF PLANT TISSUE CULTURE

- Practically any plant transformation experiment relies at some point on tissue culture. There are some exceptions to this generalisation, but the ability to regenerate plants from isolated cells or tissues *in vitro* underpins most plant transformation systems.

Plasticity and totipotency

- Two concepts, plasticity and totipotency, are central to understanding plant cell culture and regeneration.
- Plants, due to their sessile nature and long life span, have developed a greater ability to endure extreme conditions and predation than have animals. Many of the processes involved in plant growth and development adapt to environmental conditions.
- This plasticity allows plants to alter their metabolism, growth and development to best suit their environment.
- Particularly important aspects of this adaptation, as far as plant tissue culture and regeneration are concerned, are the abilities to initiate cell division from almost any tissue of the plant and to regenerate lost organs or undergo different developmental pathways in response to particular stimuli.
- When plant cells and tissues are cultured *in vitro* they generally exhibit a very high degree of plasticity, which allows one type of tissue or organ to be initiated from another type. In this way, whole plants can be subsequently regenerated.
- This regeneration of whole organisms depends upon the concept that all plant cells can, given the correct stimuli, express the total genetic potential of the parent plant. This maintenance of genetic potential is called ‘totipotency’.
- Plant cell culture and regeneration do, in fact, provide the most compelling evidence for totipotency. In practical terms though, identifying the culture conditions and stimuli required to manifest this totipotency can be extremely difficult and it is still a largely empirical process

1.1.1 Tissue culture and its history

- Plant tissue culture broadly refers to the cultivation *in vitro* of all plant parts, whether a single cell, a tissue or organ under aseptic conditions. **(PG TRB 2021)** Recent progress in the field of plant tissue culture made this area as one of the most dynamic and promising experimental biology.
- This new technique has enabled us to increase the knowledge in the following field of studies.Totipotency, nutrition, metabolism, division, differentiation and preservation of plant cells.

- Morphogenesis and plant regeneration from individual cells or tissues through the process namely organogenesis or somatic embryogenesis.
- Variations generated through *in vitro* culture.
- Evolution of haploids through anther and pollen culture including ovule culture.
- Wide hybridization programmes through ovule, ovary and embryo cultures to overcome both pre zygotic and post zygotic sterility mechanisms
- Micropropagation of plant materials
- *In vitro* selection of mutants tolerant to biotic and abiotic stresses.
- *In vitro* culture and secondary metabolite biosynthesis.

- Plant genetic engineering through *in vitro* culture methods and DNA transfer
- technique.
- Thus plant cell, tissue and organ culture permeates plant biotechnology and cements together its various aspects like Physiology, Biochemistry, Genetics and Cell Biology. Like other subjects, plant cell and tissue culture has its own origin and development.
- The chronology of major events in this field is presented for the benefit of the new entrants into this field.

HISTORY OF PLANT BIOTECHNOLOGY

1756- Duharmel du Monceau H. L discovered callus formation from the decorticated elm tree. This very old work was foreword for the discovery of plant tissue culture.

1839- Schwann, T.H expressed the view that each living cell of a multicellular organism would be capable of developing independently if provided with proper external conditions.

1853- Trecul. A performed experiment on callus formation by decorticated trees such as *Robinia, Pawlonia* and *Ulmus*.

1878- Vochting. H obtained very luxuriant callus from *Brassica rapa* and proposed the polarity in development of buds from the upper portion and roots or callus and from the lower portion of a stem piece.

1885- Roux, W.Z made the first experimental step in tissue culture when he removed a fragment of the neural plate of a chick embryo and cultivated in warm salt solution.

1893- Rechinger, C described callus formation on isolated stem fragments and root slices.

1901- Morgan, T.H coined the term totipotency to describe the capability of a cell to form an individual plant.

1902- Haberlandt, G – **Father of plant tissue culture** published a paper on "Experiments on the culture of isolated plant cells: In that he says "I should like to point out the fact that,

in my cultures, despite the conspicuous growth of the cells which frequently occurred, cell division was never observed. It will be the problem of future culture experiments to discover the condition under which isolated cells undergo division". He clearly set forth the purposes and potentialities of cell culture after having attempted and failed in the culture of isolated plant cells.

The reasons for his failure may be

(i) use of three monocotyledonous genera for much of his work,

(ii) culture of mature differentiated green mesophyll and pallisade tissues,

(iii) contamination during culture growth.

1907- Harrison, R.G devised methods of cultivating fragments of living nerve.

1910- Carrel, A was the first scientist who demonstrated the culture of living cells outside the body of an organism.

1922- Kotte, W succeeded in cultivating small root tips of pea and maize in various nutrients. The roots developed well and their growth was maintained for long periods but no subculture was attempted.

1922- Robbins, W.J started cultivation of excised root tips and stem tips of maize under sterile conditions; however, the cultures did not survive independently.

1925- Liabach, F demonstrated the most important application of the embryo culture by crossing *Linum perenae* with *L. austriacum* to get hybrid plants from shriveled seeds.

1934- Gautheret, R.J made preliminary attempts with liquid medium for cultivating plant tissues but failed completely. Later he cultured the explants on medium solidified with agar, and got healthy calli from the explants.

1934- White, P.R obtained indefinite survival of cultured tomato roots on sub culturing in liquid medium.

1939- White, P.R., Gautheret, R.J. and Nobecourt, P simultaneously announced the possibility of cultivating plant tissues for unlimited periods.

194l- Van Overbeek, J., Conklin, **M.E. and Blackeslee, A.F** established importance of coconut milk for growth and development of very young *Datura* embryos.

1942- White, P.R. and Braun, A.C initiated studies on crown gall and tumour formation in plants.

1944- Skoog, F started his work on organogenesis in tobacco callus.

1946- Ball, E.A showed development of plantlets from sterile cultures of stem tips in *Tropaeolum* and *Lupinus.* He is considered as father of micropropagation.

1947- La Rue C.D initiated endosperm cultures of *Zea mays* and obtained subcultures.

1948- Skoog, F. and Tsui, C studied the chemical control of growth and bud formation in tobacco stem segments and callus cultured *in vitro* and suggested that callus induction and shoot initiation can be regulated by making manipulations in the culture medium.

1949- Street, H.E. and Dormer, K.J initiated work on root culture and its nutrient requirements.

1951- Morel, G. and Wetmore, R.H got successful culture from monocots, once considered as recalcitrants to the cultural conditions.

1952- Steward, F.C., Caplin, S.M. and Miller, F. K discovered the synergistic action of 2, 4-D and coconut milk in a culture of potato tissue.

1952- Morel, G. and Martin, C were the first to demonstrate that virus free plants can be recovered from infected plants through shoot meristem culture.

1953- Muir, W.H found out the cultural conditions favouring the isolation and growth of single cells from higher plants *in vitro* and established nurse culture technique.

1954- Muir, W.H. Hildebrandt A.C. and Riker. A.J obtained the first suspension cultures by transferring callus fragments to agitated liquid medium

1955- Miller, C.O., Skoog, F., Von Saltza, M. and Strong, F.M identified a cell division factor *viz*., 6-furfualamino purine commonly called kinetin.

1957- Skoog, F. and Miller, C.O advanced the hypothesis that shoot and root initiation in cultured callus can be regulated by particular ratios of auxins and cytokinin.

1957- Skoog, F. and Miller, C.O discovered and introduced the idea of synergistic effects of auxins and cytokinins in promoting cell division in tobacco.

1958- Steward, F.C., Mapes, M.O. and Mears, K observed the phenomenon of somatic embryogenesis in suspension culture of carrot. They also reported that cells in suspension derived from explanted roots of cultivated carrots were capable of forming unorganised cell clusters, which in turn could yield first roots, then shoots and ultimately whole plants.

1959- Reinert, J observed the somatic embryo formation from callus cultures of carrot grown on an agarified medium.

1959- Melchers, G. and Bergmann L were first to culture haploid tissues other than pollen.

1960- Cocking, E.C discovered the technique of isolation and culture of protoplasts after digesting the cell walls enzymatically and demonstrated new cell wall regeneration on protoplasts from tomato fruit locule tissue.

1960- Bergmann L was first to obtain callus by plating cells from suspension cultures on to solid medium. This plating involved mixing cells with warm sugar medium just prior to gelation in petridish (Bergmann plating technique)

1960- Morel, G discovered a technique to produce virus free progenies by meristem culture in *Cymbidium.*

1964- Guha, S. and Maheshwari, S.C cultured mature anthers of *Datura innoxia* to study the physiology of meiosis and accidentally noticed the development of embryoids from the anthers plated on basal medium supplemented with kinetin and coconut milk.

1965- Vasil, V. and Hildebrandt, A.C described rearing of a mature tobacco plant from a single cell grown initially in microculture.

1966- Torrey, J.G advanced the hypothesis that organogenesis in callus is initiated with the formation of clusters of meristematic cells called meristemoids.

1966- Stroun, M. Anker, P., Charles, P. and Le Doux L made DNA transfer in tomato under *in vitro* conditions.

1970- Kasha, K. J and Kao, K.N produced haploid plants of *Hordeum vulgare* by *in vitro* culturing of embryos obtained by cross *Hordeum vulgare* with *Hordeum bulbosum* in which elimination of *bulbosum* chromosome occurred.

1971- Takebe, I., Labib, G. and Melchers, G regenerated whole plants from isolated mesophyll protoplasts of tobacco.

1971- Bendich, A.J. and Filner, P used the cells and tissues in culture for transformation studies.

1972- Withers, L. and Cocking, E.C laid foundation for the protoplast fusion technique.

1973- Potrykus, I made the first attempt on chloroplast and nucleus transfer from *Petunia hybrida* into albino protoplasts of the same species.

1974- Melchers, G. and Labib, G proposed hybrids resembling the sexual hybrids by fusing protoplasts of two varieties of *Nicotiana tabacum.*

1974- Murashige, T developed the concept of developmental stages in cultures *in vitro*:
Stage I: Explant establishment;

Stage II: Multiplication of propagule and

Stage III: Rooting and hardening for planting into soil.

1975- Morel, G established cold storage of regenerated plants for a year.

1976- Mullin, R.H. and Schlegal, D.E successfully employed cold storage to maintain *in vitro* virus free plantlets of strawberry.

1976- Seibert, M established shoot initiation from carnation shoot apices frozen to -196xC.

1978- Zelcer, A., Aviv, D. and Galun E developed a protoplast fusion procedure called Donor - Recipient protoplast fusion to favour organelle transfer among plants.

1979- Polacco, J.C. Sparks, R.B. and Havir, E.A described the cloning of soyabean urease structural gene by the vector mediated transfer system.

1980- Gleba Y. Y. and Hoffmann F synthesized a new plant "*Arabidobrassica* by fusing the protoplasts *Arabidopsis* and *Brassica*.

1981- Larkin, P.J. and Scowcroft, W.R developed the concept of somaclonal variation: A noval source of variability from cell cultures for plant improvement.

1981- Patnaik, G., Wilson, D. and Cocking, E.C regenerated a whole plant from a single free cultured tobacco protoplast.

1982- Krens, F.A., Molondijk, L. Williams G. J. and Schilperoort, R.A developed poly ethylene glycol method for the direct delivery of DNA into protoplasts.

1983- Zambryski, P. Joos, H., Genetello, C., Leemans, J. Van Montagu M. and Schell Constructed Ti plasmid vector for the introduction of DNA into plant cells without alteration of their normal regeneration capacity.

l984- Watts , J. W. and King, J. M developed a simple method for large scale electrofusion of protoplasts.

l984- Brisson, N. Paszkowski, J. Penswick, J. R. Gronenborn, B. Potrykus, I. and Hohn, T Achieved transformation in which part of the cauliflower mosaic virus genome was replaced by selectable marker.

1985- Gheysen, G. Dahese, P., Van Montaque, M. and Schell, J developed very efficient gene transfer system using natural gene transfer mechanism of *Agrobacterium tumifaciens.*

1985- Cocking E. C exposed plasma membrane in the tips of root hairs of wide range of crop plants. The procedure enabled whole seedlings to have the plasma membrane at the tips of their root hairs exposed to foreign DNA and other microorganisms.

1985- Tabata, M. and Fujita, Y developed the technique of elucidation of the physical and chemical factors controlling the biosynthesis of the red napthoquinone pigments by *Lythospermum erythrorhizon*.

1986- Crossway, A. Oakes, J.V., Irvine , J.M., Ward B. Knauf, V.C. and Shewmaker, C.K developed a direct way of transferring cloned genes into protoplasts by microinjection of DNA directly into the nucleus of tobacco mesophyll protoplasts.

1986-Hamill, J. D. Parr, A. J., Robins, R. J. and Rhodes, M.J.C established hairy root cultures of *Beta vulgaris* and *Nicotinna rustica* following infection with *Agrobacterium rhizogenes* and the transformed cultures synthesized their characteristic secondary

products at levels comparable with those of *in vitro* roots from the same variety.

1986- Abdullah, R., Cocking, E.C., and Thompson, J.A demonstrated that normal green rice plants can be regenerated efficiently and reproducibly from rice protoplasts *via* Somatic embryogenesis.

1986- Pirrie, A. and Power, J.B produced fertile, interspecific gametosomatic triploid hybrids of tobacco by fusing protoplasts of leaf (2n) and pollen tetrad (n).

1986- Kinsara A., Patnaik, S.N., Cocking, E.C. and Power, J.B produced somatic hybrids between *Lycopersicon esculentum* and *L. peruvianum*.

1987- Terada, R., Kyozuka, J., Nishibayashi, S., and Shimamoto, K regenerated plantlets form somatic hybrid cells of *Oryza sativa*, and *Echinochloa oryzicola*.

1987- Ethlenfelt, N.K. and Helgeson, J.P produced tetroploid and hexaploid somatic hybrids from protoplast fusions between *Solanum bravidens* (2x, non tuber bearing species) and 2x and 4x *S. tuberosum*.

1987- Neuhaus, G., Spangenberg, G. Mittelsten Sheid, O and Schweiger, H.G effected gene transfer by microinjecting the DNA into the cells of microspore derived proembryos.

1987- De la Pe$a, A., Lornz, H., Schell, J developed transgenic rye plants obtained by injecting DNA into young floral tillers.

1988- Nomoru, K. and Komamine, A used single cells of carrot from a cell suspension instead of protoplasts, for microinjection and the microinjected carrot cells could divide and differentiated to embryos at a frequency of about 50 percent.

1988- Rhodes, C.A., Pierce, D.A., Mettler, I. J.. Mascarenhas, D. and Detmer J.J produced transgenic maize plant by electroporation.

1988- Toriyama, K., Arimoto, Y. Uchimiya, H, and Hinata K produced transgenic rice plant by electroporation.

1989- Shimamoto, K., Terada, R., Izawa, T. and Fujimoto, H produced fertile transgenic rice plants regenerated from transformed protoplasts.

1989- Prioli, L. M. and Sondahl, M. R recovered fertile plants from protoplasts of maize.

1990- Milanova, V. and Zagorska, N. A succeeded in overcoming hybrid incompatibility between *Nicotiana africana* and *N. tabacum* and produced cytoplasmic male sterile plants by embryo culture.

1990- Iida, A., Seki, M. Kamada, M. YHamada Y. and Morikawa delivered genes into cultured plant cells by DNA-coated gold particles accelerated by a pneumatic particle gun.

1991- Kyozuka, J. Fujimoto, H., Izawa, T. and Shimamoto- K succeeded in getting tissue specific expression of maize alcohol dehydrogenase l gene in transgenie rice plants and their progenies.

1991- Spangenberg, G., Fredyl, E., Osusky, M. Nagel, J. and Potrykus, I developed a method for the predictable transfer of partial genomes predictable transfer of partial genomes by using sub protoplasts (cytoplasts and karyoplasts).

1991- Sautter, C., Waldner, H. Neuhaus, G., Galli, A. Neuhaus, G. and Potrykus developed a novel method for the acceleration of micro projectiles. The method is called as micro targeting.

The history of plant tissue culture had its real beginning in 1934 when Gautheret tried to cultivate isolated cells and root tips on organised medium. The momentum from this pioneering work, a new turn in this ongoing research occurred, because of World War II.

After the Second World War, American plant pathologists became interested in plant tissue culture.As **Steward (1970)** pointed out, the plant tissue culture technique is another "**Silent Revolution in Agriculture**" having very good potentials to supplement conventional breeding approaches. Its potentials and prospects are discussed in subsequent chapters. The techniques' theoretical aspects and their applicabilities are simplified and presented.

1.1.2. EXERCISE

1.Callus formation in the decorticated elm tree was discovered by ……..

A) Duharmel du Monceau B) Morgan, T.H

C) Haberlandt D) d) Rechinger

2. Who's work was foreword for the discovery of plant tissue culture?.

A) Duharmel du Monceau B) Morgan, T.H

C) Haberlandt D) Rechinger

3. Name the scientist who expressed the view that each living cell of a multicellular organism would be capable of developing independently if provided with proper external conditions

A) Duharmel du Monceau B) Schwann

C) Haberlandt D) Rechinger

4. Name the scientist who proposed the polarity in development of buds from the upper portion and roots or callus and from the lower portion of a stem piece.

A) Duharmel du Monceau B) Morgan, T.H

C) Haberlandt D) Vochting

5. Callus formation on isolated stem fragments and root slices is described by …….

A) Duharmel du Monceau B) Morgan, T.H

C) Haberlandt D) Rechinger

6. The term totipotency was coined by ……..

A) Duharmel du Monceau B) Morgan, T.H

C) Haberlandt D) Rechinger

7. The term totipotency means ……..

A) the capability of a cell to form an individual plant

B) the capability of a cell to form an individual cell

C) both a & b

D) None of the above

8. Father of plant tissue culture is ……..

A) Duharmel du Monceau B) Morgan, T.H

C) Haberlandt D) Rechinger

9. The embryo culture was first demonstrated by ……..

A) Robbins B) Harrison C) Liabach D) Kotte

10. Father of micropropagation is ……..

A) Ball B) Harrison C) Robbins D) Kotte

1.2 NUTRIENTS FOR PLANT CELLS

Plant Nutrition is the study of the chemical elements that are necessary for growth. In 1972, E. Epstein defined 2 criteria for an element to be essential for plant growth:

1. in its absence the plant is unable to complete a normal life cycle or
2. that the element is part of some essential plant constituent or metabolite,

This is all in accordance with Liebig's law of the minimum. There are 17 essential plant nutrients. Carbon and oxygen are absorbed from the air, while other nutrients including water are obtained from the soil.

Plants must obtain the following mineral nutrients from the growing media:

- The primary macronutrients: nitrogen (N), phosphorus (P), potassium (K)
- The three secondary macronutrients such as calcium (Ca), sulphur (S), magnesium (Mg).
- The macronutrient Silicon (Si) And micronutrients or trace minerals: boron (B), chlorine (Cl), manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), molybdenum (Mo), nickel (Ni), selenium (Se), and sodium (Na).

Plant cell culture media(PG TRB 2003-2004)

Culture media used for the cultivation of plant cells in vitro are composed of three basic components:

1 essential elements, or mineral ions, supplied as a complex mixture of salts;

2 an organic supplement supplying vitamins and/or amino acids; and

3 a source of fixed carbon; usually supplied as the sugar sucrose.

1.2.1 Types Of Tissue Culture Media

Many types of media are modifications of Murashige and Skoog salt base. Other commonly used media includeGamborg B5, Lloyd and McCown woody plant, Nitsch and Nitsch, Schenk and Hildebrandt, and White salt basal medium.

Complex mixture of salts: These include essential elements or mineral ions important for plant nutrition and their physiological function. The essential elements can further be divided into the following categories:

a. Macroelements (or macronutrients)
b. Microelements (or micronutrients)
c. Iron source

TCA

Macro nutrients

- As is implied by the name, the stock solution supplies macro elements required in large amounts for plant growth and development.
- Nitrogen, phosphorus, potassium, magnesium, calcium, and sulphur (and carbon, which is added separately) are usually regarded as macroelements.
- These elements usually comprise at least 0.1% of the dry weight of plants. Nitrogen is most commonly supplied as a mixture of nitrate ions (from KNO_3) and ammonium ions (from NH_4NO_3).
- Theoretically, there is an advantage in supplying nitrogen in the form of ammonium ions, as nitrogen must be in the reduced form to be incorporated into macromolecules.
- Nitrate ions therefore need to be reduced before incorporation. However, at high concentrations, ammonium ions can be toxic to plant cell cultures and uptake of ammonium ions from the medium causes acidification of the medium.
- For ammonium ions to be used as the sole nitrogen source, the medium needs to be buffered. High concentrations of ammonium ions can also cause culture problems by increasing the frequency of vitrification (the culture appears pale and ‘glassy’ and is usually unsuitable for further culture).
- Using a mixture of nitrate and ammonium ions has the advantage of weakly buffering the medium as the uptake of nitrate ions causes OH^- ions to be excreted. Phosphorus

is usually supplied as the phosphate ion of ammonium, sodium, or potassium salts. High concentrations of phosphate can lead to the precipitation of medium elements as insoluble phosphates.

Macro nutrients

Carbon

- Carbon forms the backbone of many plants biomolecules, including starches and cellulose.
- Carbon is fixed through photosynthesis from the carbon dioxide in the air and is a part of the carbohydrates that store energy in the plant.

Hydrogen

- Hydrogen also is necessary for building sugars and building the plant. It is obtained almost entirely from water.
- Hydrogen ions are imperative for a proton gradient to help drive the electron transport chain in photosynthesis and for respiration.

Oxygen

- Oxygen is necessary for cellular respiration. Cellular respiration is the process of generating energy-rich adenosine triphosphate (ATP) via the consumption of sugars made in photosynthesis.
- Plants produce oxygen gas during photosynthesis to produce glucose but then require oxygen to undergo aerobic cellular respiration and break down this glucose and produce ATP.

Phosphorus

- Phosphorus is important in plant bioenergetics. As a component of ATP, phosphorus is needed for the conversion of light energy to chemical energy (ATP) during photosynthesis.
- Phosphorus can also be used to modify the activity of various enzymes by phosphorylation, and can be used for cell signaling.

Nitrogen

- Nitrogen is an essential component of all proteins.
- Nitrogen deficiency most often results in stunted growth, slow growth, and chlorosis**. (CSIR NET DEC 2012)**
- Nitrogen deficient plants will also exhibit a purple appearance on the stems, petioles and underside of leaves from an accumulation of anthocyanin pigments earlier than the younger leaves. Soluble forms of nitrogen are transported as amines and amides.

Sulphur

- Sulphur is a structural component of some amino acids and vitamins, and is essential in the manufacturing of chloroplasts.
- Sulphur is also found in the Iron Sulphur complexes of the electron transport chains in photosynthesis. It is immobile and deficiency therefore affects younger tissues first.
- Symptoms of deficiency include yellowing of leaves and stunted growth.

Calcium

- Calcium regulates transport of other nutrients into the plant and is also involved in the activation of certain plant enzymes.
- Calcium deficiency results in stunting.

Magnesium

- Magnesium is an important part of chlorophyll, a critical plant pigment important in photosynthesis.
- It is important in the production of ATP through its role as an enzyme cofactor.
- There are many other biological roles for magnesium—Magnesium in biological systems for more information. Magnesium deficiency can result in interveinal chlorosis.

Silicon

- In plants, silicon strengthens cell walls, improving plant strength, health, and productivity.
- Other benefits of silicon to plants include improved drought and frost resistance, decreased lodging potential and boosting the plant's natural pest and disease fighting systems.
- Silicon has also been shown to improve plant vigor and physiology by improving root mass and density, and increasing above ground plant biomass and crop yields.

Micro nutrients

- Microelements are required in trace amounts for plant growth and development,and have many and diverse roles.
- Manganese, iodine, copper, cobalt, boron, molybdenum,iron, and zinc usually comprise the microelements, although other elementssuch as nickel and aluminium are found frequently in some formulations.
- Iron is usually added as iron sulphate, although iron citrate can also be used.Ethylenediaminetetra-acetic acid (EDTA) is usually used in conjunction with ironsulphate.
- The EDTA complexes with the iron to allow the slow and continuous release of iron into the medium. Uncomplexed iron can precipitate out of the medium as ferric oxide.

- Some elements are directly involved in plant metabolism (Arnon and Stout, 1939) However, this principle does not account for the so-called beneficial elements, whose presence, while not required, has clear positive effects on plant growth.
- Mineral elements which either stimulate growth but are not essential or which are essential only for certain plant species, or under given conditions, are usually defined as beneficial elements.

Iron

- Iron is necessary for photosynthesis and is present as an enzyme cofactor in plants.
- Iron deficiency can result in interveinal chlorosis and necrosis.

Molybdenum

- Molybdenum is a cofactor to enzymes important in building amino acids.

Boron

- Boron is important for binding of pectins in the RGII region of the primary cell wall, secondary roles may be in sugar transport, cell division, and synthesizing certain enzymes.
- Boron deficiency causes necrosis in young leaves and stunting.

Copper

- Copper is important for photosynthesis. Symptoms for copper deficiency include chlorosis. Involved in many enzyme processes.
- Necessary for proper photosythesis. Involved in the manufacture of lignin (cell walls). Involved in grain production.

Manganese

- Manganese is necessary for building the chloroplasts. Manganese deficiency may result in coloration abnormalities, such as discolored spots on the foliage.

Sodium

- Sodium is involved in the regeneration of phosphoenolpyruvate in CAM and C4 plants. It can also substitute for potassium in some circumstances.

Zinc

- Zinc is required in a large number of enzymes and plays an essential role in DNA transcription.
- A typical symptom of zinc deficiency is the stunted growth of leaves, commonly known as "little leaf" and is caused by the oxidative degradation of the growth hormone auxin.

Nickel

- In higher plants, Nickel is essential for activation of urease, an enzyme involved with nitrogen metabolism that is required to process urea.

- Without Nickel, toxic levels of urea accumulate, leading to the formation of necrotic lesions. In lower plants, Nickel activates several enzymes involved in a variety of processes, and can substitute for Zinc and Iron as a cofactor in some enzymes.

Chlorine

- Chlorine is necessary for osmosis and ionic balance; it also plays a role in photosynthesis.

Cobalt

- Cobalt has proven to be beneficial to at least some plants, but is essential in others, such as legumes where it is required for nitrogen fixation for the symbiotic relationship it has with nitrogen-fixing bacteria

Element Function

s.no	Element	Function
1	Nitrogen	Component of proteins, nucleic acids, and some coenzymes; element required in the greatest amounts
2	Potassium	Regulates osmotic potential; principal inorganic cation
3	Calcium	Cell-wall synthesis, membrane function, cell signalling
4	Magnesium	Enzyme cofactor, component of chlorophyll
5	Phosphorus	Component of nucleic acids; energy transfer; component of intermediates in respiration and photosynthesis
6	Sulphur	Component of some amino acids (methionine, cysteine) and some cofactors
7	Chlorine	Required for photosynthesis
8	Iron	Electron transfer as a component of cytochromes
9	Manganese	Enzyme cofactor
10	Cobalt	Component of some vitamins
11	Copper	Enzyme cofactor; electron-transfer reactions
12	Zinc	Enzyme cofactor; chlorophyll biosynthesis
13	Molybdenum	Enzyme cofactor; component of nitrate reductase

Composition of a typical plant culture medium

TCA	**Essential element**	**Concentration in stock solution (mg l−1)**	**Concentration in medium (mg l−1)**
A	**Macroelements**		
1	NH4NO3	33 000	1 650
2	KNO3	38 000	1 900
3	CaCl2·2H2O	8 800	440
4	MgSO4·7H2O	7 400	370
5	KH2PO4	3 400	170
B	**Microelements**		
1	KI	166	0.83
2	H3BO3	1 240	6.2
3	MnSO4·4H2O	4 460	22.3
4	ZnSO4·7H2O	1 720	8.6
5	Na2MoO4·2H2O	50	0.25
6	CuSO4·5H2O	5	0.025
7	CoCl2·6H2O	5	0.025
C	**Iron source**		
1	FeSO4·7H2O	5 560	27.8
2	Na2EDTA·2H2O	7 460	37.3
D	**Organic supplement**		
1	Myoinositol	20 000	100
2	Nicotinic acid	100	0.5
3	Pyridoxine -HCl	100	0.5
4	Thiamine-HCl	100	0.5
5	Glycine	400	2
E	**Carbon source**		
1	Sucrose	Added as solid	30 000

இன்றைய *TRB* பயிற்சியாளரே நாளைய அரசு பள்ளி ஆசிரியரே!

Teacher's Care Academy **கடந்த *14* ஆண்டுகளாக *TRB* தேர்வுகளுக்கான சிறப்பு பயிற்சியை வழங்கி வருகிறது. இதுவரை *10,000*-க்கும் மேற்பட்ட ஆசிரியர்களை அரசு வேலைகளில் வெற்றிகரமாக நியமிக்க உதவியதில் நாங்கள் பெருமிதம் கொள்கிறோம். எங்கள் நிறுவனத்தில் அனைத்து *TRB* தேர்வுகளுக்கும் விரிவான பயிற்சிகள் உள்ளன, அவை:**

- ***PGTRB***
- ***UGTRB***
- ***SGT***
- ***POLYTECHNIC TRB***
- ***BEO***
- ***TET Paper I & II***
- ***College TRB***
- ***Special Teachers***

கூடுதலாக, தமிழ்நாடு அரசு இப்போது அனைத்து அரசு பணிக்கான தேர்வாணையங்களுக்கு *(TRB, TNPSC, MRB, TNUSRB)* தமிழ் மொழி கட்டாய தகுதி தேர்வு *(Tamil Compulsory Exam)* முதற்கட்ட தேர்வாக அறிவித்துள்ளது இதற்காக தமிழ் மொழி கட்டாய தகுதி தேர்வு என்ற புத்தகத்தை பிரத்தியேகமாக உங்கள் *Teacher's Care Academy* வெளியிட்டுள்ளது. இந்த புத்தகம் அமேசானிலும் கிடைக்கிறது ஆனால் எங்களை நேரடியாக தொடர்பு கொண்டு வாங்கும் போது உங்களுக்கு கூடுதல் தள்ளுபடி கிடைக்கும்

PGTRB

PGTRB தேர்விற்கு நாங்கள் அனைத்து மொழி பாடத்திற்கும் பயிற்சிகளை வழங்கி வருகிறோம் அதாவது

- ***Tamil***
- ***English***
- ***Mathematics***
- ***Physics***
- ***Chemistry***
- ***Botany***
- ***Zoology***
- ***Economics***
- ***Commerce***
- ***Computer Science***
- ***History***

மேற்கண்ட அனைத்து படப்பிரிவுகளுக்கான ***Study Material***-களுடன் ***Psychology, Tamil Eligibility Book, Question Bank*** மற்றும் ***General Knowldge Material***-களும் வழங்கப்படும்

TET (Teachers Eligibility Test)

TET தேர்விற்கு நம் ***Teachers Care Academy***-யில் ***Paper I*** மற்றும் ***Paper II*** என இரண்டு தாள்களுக்கும் பிரத்தியேகமாக பயிற்சிகளை வழங்குகிறோம்

இதற்கு தமிழ்நாடு அரசால் வழங்கப்பட்டுள்ள பள்ளி பாட புத்தகத்தில் இருந்து குறிப்புகளை எடுத்து ***Study Material***-களாக வழங்குகிறோம்

மேலும் ***Psychology***-க்கு ***TRB***-ஆல் வழங்கப்பட்டுள்ள பாடத்திட்டத்தை பின்பற்றி பல்வேறு ***Reference Book***-லிருந்து குறிப்புகளை எடுத்து ***Study Material***-களாக வழங்குகிறோம்

UGTRB

TET தேர்வில் வெற்றி பெற்ற ஆசிரியர்களுக்கு நடத்தப்படும் ***UGTRB*** போட்டி தேர்வுக்காக அனைத்து மொழி பாடத்திற்கும் பயிற்சிகளை வழங்கி வருகிறோம் அதாவது

- ***Tamil***
- ***English***
- ***Mathematics***
- ***Physics***
- ***Chemistry***
- ***Botany***
- ***Zoology***
- ***History***
- ***Geography***

SGTRB

TET தேர்வில் வெற்றி பெற்ற ஆசிரியர்களுக்கு நடத்தப்படும் ***SGTRB*** போட்டி தேர்வுக்காக தமிழ்நாடு அரசால் வழங்கப்பட்டுள்ள பள்ளி பாட புத்தகத்தில் இருந்து குறிப்புகளை எடுத்து ***Study Material***-களாக வழங்குகிறோம்

BEO

BEO தேர்வுக்காக ***TRB***-ஆல் பாடத்திட்டம் வெளியிடப்பட்டுள்ளது அந்த பாடத்திட்டத்தின் அடிப்படையில் அனைத்து பாடத்திற்கும் உங்கள் ***Teachers Care Academy*** அலகு ***(Unit-Wise)*** வாரியாக ***Study Material***-களை வழங்குகிறது.

POLYTECHNIC TRB

Polytechnic தேர்விற்காக உங்கள் ***Teachers Care Academy*** பின்வரும் மொழி பாடத்திற்கு பயிற்சிகளை வழங்கி வருகிறது. அதாவது,

- ***Civil***
- ***EEE***
- ***ECE***
- ***CSE***
- ***Mechanical***
- ***English***
- ***Mathematics***
- ***Physics***
- ***Chemistry***

College TRB

தமிழ்நாட்டில் அரசு கல்லூரிகளில் காலியாக உள்ள உதவி பேராசிரியர் பணிக்கு ***TRB*** வெகு விரைவில் போட்டித் தேர்வை நடத்த இருக்கிறது

அந்த தேர்வுக்காக நம் ***Teachers Care Academy***-யில் பின்வரும் மொழி பாடத் திட்டத்திற்கும் பயிற்சிகளை வழங்கி வருகிறது

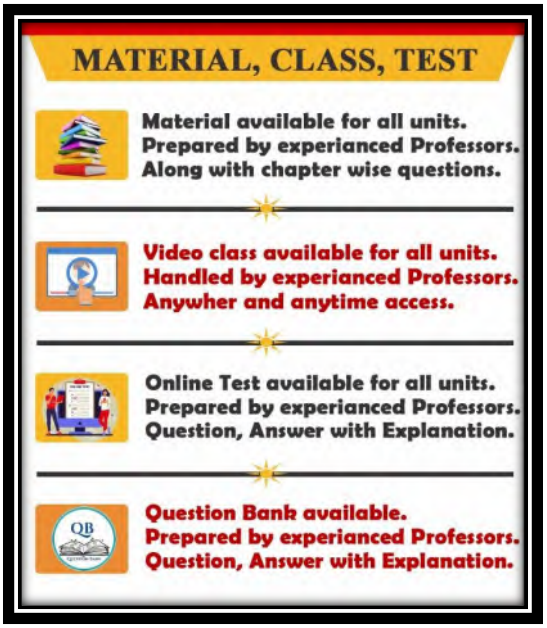
- ***Tamil***
- ***English***
- ***Mathematics***
- ***Physics***
- ***Chemistry***
- ***Botany***
- ***Zoology***
- ***Economics***
- ***Commerce***
- ***Computer Science***
- ***History***
- ***Geography***

Special Teachers

TRB-ஆல் நடத்தப்படும் சிறப்பாசிரியர் தேர்வுக்காக நம் Teachers Care Academy-யில் பின்வரும் பாடத்திட்டத்திற்கு பிரத்தியேகமாக பயிற்சிகள் வழங்கப்பட்டு வருகிறது. அதாவது,

- ***Sewing***
- ***Drawing***
- ***Music***
- ***PET***

மேற்கண்ட அனைத்து தேர்வுகளுக்கும் உங்கள் Teachers Care Academy பலவிதமான பயிற்சிகளை வழங்குகிறது, அவை

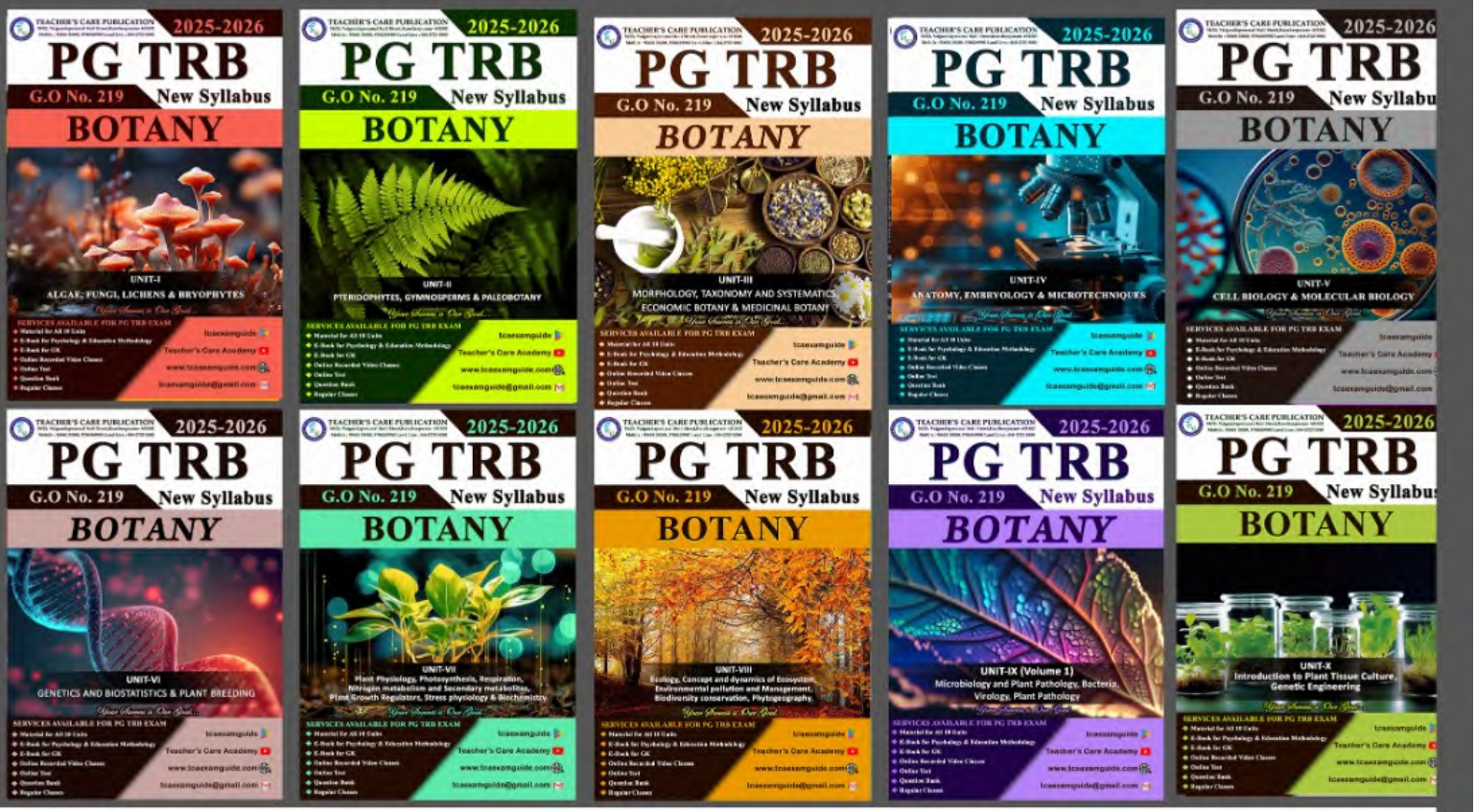


இந்த ஆண்டு (2024) TNPSC Batch-யும் அறிமுகம் செய்திருப்பதில் நாங்கள் பெருமிதம் கொள்கிறோம். எங்கள் வழிகாட்டுதல் வரவிருக்கும் தேர்வுகளில் நீங்கள் வெற்றி பெற உதவும் என நாங்கள் உறுதியாக நம்புகிறோம்.

உங்கள் அரசு ஆசிரியர் பணி கனவு நிறைவேற வாழ்த்துக்கள்!

அன்புடன்,

Teacher's Care Academy



Teachers care Academy is the foremost coaching Institution for various competitive examinations such as P.G.TRB, TET Papers I &II TNPSC including special Teachers Eligibility Test. The Academy was established on 5th April 2013 by learned energetic and multifaceted chairperson Mrs. RAVIMAGESHWARI in the holy temple town of Kanchipuram.

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- Block Educational Officer
- Teachers Eligibility Test (TET Paper-1 & Paper-2) & UG-TRB
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- TNPSC (Group-1, Group-2, Group-3, Group-4)
- NEET

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