Light and Electron microscopy (TEM & SEM), Confocal and phase contrast microscopy,.

Year	***Scientist***	***Contribution***
1590	*Zacharias Janssen*	*Developed the first compound microscope.*
1590	*Robert Hooke*	*Observed nonliving plant tissue of a thin slice of cork.*
1668	*Francesco Redi*	*Discovered that microorganism did not spontaneously appear. His contribution led to the finding that killing the microorganism that caused the disease could prevent the disease.*
1673	*Antoni van Leeuwenhoek*	*Invented the single-lens microscope, grinding the microscope lens to improve magnification. First person to view a living organism.*
1798	*Edward Jenner*	*Developed vaccinations against disease-causing microorganisms.*
1850s	*Mathias Schleiden, Theodore Schwann, Rudolf Virchow*	*Developed cell theory.*
1847	*Ignaz Semmelweis*	*Reported a dramatic decline in childbirth fever after physicians used antiseptic techniques when delivering babies.*
1864	*Louis Pasteur*	*Discovered that microorganisms were everywhere, living on organisms and in nonliving things such as air. His work led to improved sterilization techniques called pasteurization. One of the founders of bacteriology.*
1867	*Joseph Lister*	*Reduced infections after surgery by spraying carbolic acid over the patient before bandaging the wound. This was the first surgical antiseptic.*
1876	*Robert Koch*	*Discovered how microorganisms spread contagious diseases by studying anthrax. Developed the Germ Theory. Developed techniques for cultivating microorganisms.*
1870s	*John Tyndall, Ferdinand Cohn*	*Discovered that some microorganisms are resistant to certain sterilization techniques. One of the founders of bacteriology.*
1884	*Elie Metchnikoff*	*Discovered that white blood cells (*leukocytes*) engulf and digest microorganisms that invade the body. Coined the word* phagocytes*. Founded the branch of science called immunology.*
1887	*Richard Petri*	*Developed the technique of placing agar into a specially designed dish to grow microorganisms, which was later called the Petri Dish.*
1890	*Paul Ehrlich*	*Developed the first drug to fight disease-causing microorganisms that had already entered the body.*
1928	*Alexander Fleming*	*Discovered* Penicillium notatum*, the fungus that kills* staphylococcus aureus*, a microorganism that is a leading cause of infection.*

MICROSCOPIC METHOD

The microscope is the instrument used in most of the microbiology laboratory. The magnification it provides enables us to see microorganisms and their structures which are otherwise invisible to the naked eye. The magnifications attainable by microscopes range from 100 X to 400,000 X.

Microscopes are of two categories: (1) Light or optical Microscopes and (2) Electron Microscopes.

(1) Light microscopy: *in which magnification is obtained by a system of optical lenses using light waves, includes:*

i. Bright-field microscopy
ii. Dark-field microscopy
iii. Fluorescence microscopy
iv. Phase-contrast microscopy
v. Differential interference contrast (DIC) microscope
vi. Confocal microscopy

i. Bright-field microscopy: *In bright-field microscopy, the microscopic field is brightly lighted and the microorganisms appear dark because they absorb some of the light. Ordinarily, microorganisms do not absorb much light, but staining them with a dye greatly increases their light-absorbing ability resulting in greater contrast and color differentiation. Generally microscopes of this type produce a useful magnification of about 1,000 X to 2, 000 X. The basic limitation of the bright-field microscope is one not of magnification but of* ***resolving power(R.P.), the ability to distinguish two adjacent points as distinct and separate.***

⇨*The angle* θ *subtended by the optical axis and the outermost rays still covered by the objective is the measure of the aperture of the objective; it is the half-aperture angle.*

$$NA = n \sin \theta$$

The limit of resolution is the smallest distance by which two objects can be separated and still be distinguishable as two separate objects. The greatest resolution in light microscopy is obtained with the shortest wavelength of visible light and an objective with the maximum NA.

$$D = \frac{.61\,\lambda}{n \sin\theta}$$

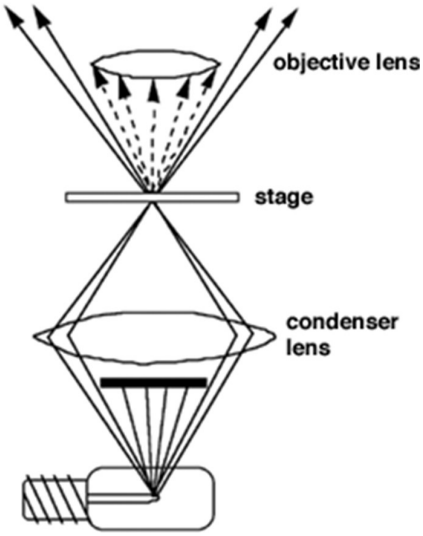
- *R.P. is smallest for violet light, but because human eye is more sensitive to blue, optimal R.P. is achieved with blue light (~450 nm). Use filters to remove other light in best microscopes*
- *n sinØ is called* ***numerical aperture****. It measures how much light cone spreads out between condenser & specimen.*
- *More spread = better resolution. Ø = angle of light cone; maximum value is 1.0*
- *n = refractive index. n = 1.0 in air. Can increase with certain oils (up to 1.4), called* ***immersion oil.*** *N.A. is property of lens. Look on side of lens to identify.*
- *Theoretical limit of R.P. for light scope is 0.2 micrometers.*

Optical Instrument	***Resolving Power***	***RP in Angstroms***
Human eye	*0.2 millimeters (mm)*	*2,000,000 Å*
Light microscope	*0.20 micrometers (µm)*	*2000 Å*
Scanning electron microscope (SEM)	*5-10 nanometers (nm)*	*50-100 Å*
Transmission electron microscope (TEM)	*0.5 nanometers (nm)*	*5 Å*

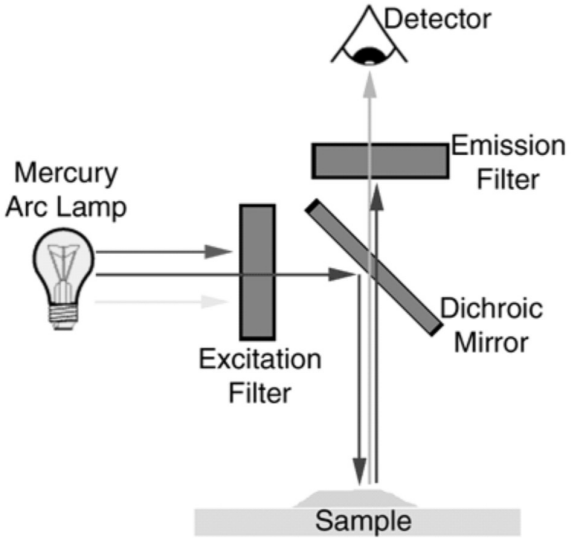
Most laboratory microscopes are equipped with three objectives, each capable of a different degree of magnification. ***These are referred to as the oil-immersion, high-dry, and low-power objectives****.*

ii. Dark-field Microscopy: *The effect produced by the dark-field technique is that of a dark background against which objects are brilliantly illuminated. This is accomplished by equipping the light microscope with a special kind of*

condenser that transmits a hollow cone of light from the source of illumination. This diffracted light will enter the objective and reach the eye; thus the object or microbial cell, in this case, will appear bright in an otherwise dark microscopic field.



Dark Field Microscopy



Fluorescence Microscopy

iii. Fluorescence Microscopy: *In microscopy, fluorescence can be used as a label or tag when preparing specific biological probes. Some biological substances, such as chlorophyll and some oils and waxes, have primary fluorescence (auto-fluorescence). But most biological molecules or structures do not fluorescence on their own, so they must be linked with fluorescent molecules,* ***fluorochromes,*** *in order to create specific fluorescent probes.*

- *The fluorochromes emit light of a given wavelength when excited by incident light of a different (shorter) wavelength. To view this fluorescence in the microscope, several light filtering components are needed. Specific filters are used to isolate the excitation and emission wavelengths of a fluorochrome. A dichroic beam splitter (partial mirror) reflects shorter wavelengths of light and allows longer wavelengths to pass. A dichoric beam splitter is required because the objective acts as both a condenser lens (excitation light) and objective lens (emission light); therefore the beam splitter isolates the emitted light from the excitation wavelength.*
- *This epi-illumination type of light path is required to create a dark background so that the fluorescence can be easily seen.*
- *The wavelength at which a beam splitter allows the longer wavelengths to pass must be set between the excitation and emission wavelengths of any given fluorochrome so that excitation light is reflected and emission light is allowed to pass through it.*
- *A bright light source producing the correct wavelengths for excitation is also required for fluorescence microscopy, normally a mercury arc lamp. When using confocal microscopy to view fluorescence, where up to 95% of the emission light is filtered out, specific wavelength lasers are used, as these are extremely bright and monochromatic. Fluorescent labelling has a further advantage over other histochemical stains, in that two or more fluorochromes can be detected separately using optical filters, therefore components can be labelled specifically and identified separately in the same sample (double labelling).*

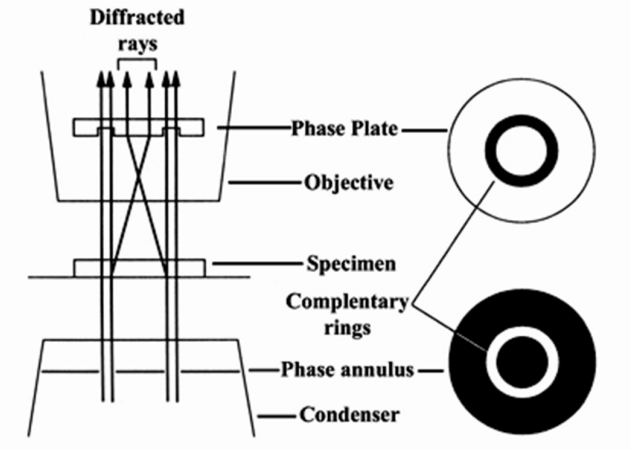
⇨TYPES OF FLUOROPHORES USED in fluorescence micro scopy: -

- ❖ fluorescein,
- ❖ DAPI,
- ❖ propidium iodide,
- ❖ green fluorescent protein (GFP),
- ❖ Texas Red

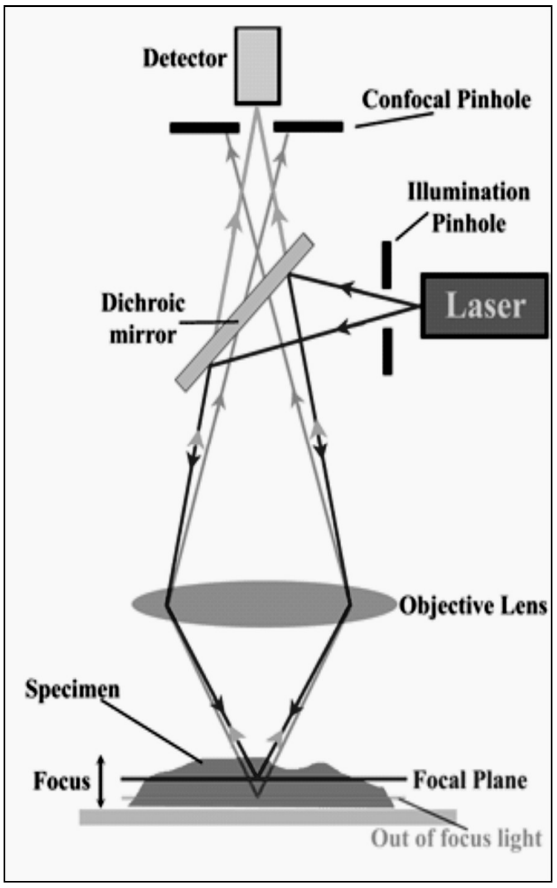
iv. Phase-contrast microscopy: ***Phase contrast is a method used in microscopy and developed in the early 20th century by Frits Zernike*** *Phase-contrast microscopy is extremely valuable for studying living unstained cells and is applied and theoretical biological studies. It uses a conventional light microscope fitted with a phase-contrast objective and a phase-contrast condenser. In principle, this technique is based on the fact that light passing through one material and into another material of a slightly different refractive index and/or thickness will undergo a change in phase.*

⇨*Highly refractive structures bend light to a much greater angle than do structures of low refractive index. The same properties that cause the light to bend also delay the passage of light by a quarter of a wavelength or so.*

⇨ *These differences in phase, or wave-front irregularities, are translated into variations in brightness of the structures and hence are detectable by the eye (The degree of reduction in brightness depends on the refractive index of the object.).* ***With phase-contrast microscopy it is possible to reveal differences in cells and their structures not discernible by other microscopic methods****.*



v. Differential interference contrast (DIC) microscope *Like the phase-contrast microscope, the differential interference contrast (DIC) microscope provides a detailed view of unstained, live specimens by manipulating the light. But this microscope has additional refinements, including two prisms that add contrasting colors to the image and two beams of light rather than a single one. DIC microscopes produce extremely well-defined images that are vividly colored and appear three-dimensional*



vi: Confocal microscopy

Although conventional light and fluorescence microscopy allow the examination of both living and fixed specimens, certain problems exist with these techniques. One of the main problems is out-of-focus blur degrading the image by obscuring important structures of interest, particularly in thick specimens. In conventional microscopy, not only is the plane of focus illuminated, but much of the specimen above and below this point is also illuminated resulting in out-of-focus blur from these areas. This out-of-focus light leads to a reduction in image contrast and a decrease in resolution. In the confocal microscope all out-of-focus structures are suppressed at image formation. This is obtained by an arrangement of diaphragms, which, at optically conjugated points of the path of rays, act as a point source and as a point detector respectively. The detection pinhole does not permit rays of light from out-of-focus points to pass through it. The wavelength of light, the numerical aperture of the objective and the diameter of the diaphragm (wider detection pinhole reduces the confocal effect) affect the depth of the focal plane. To obtain a full image, the point of light is moved across the specimen by scanning mirrors. The emitted/reflected light passing through the detector pinhole is transformed into electrical signals by a photomultiplier and displayed on a computer monitor. **Confocal microscopy**

- Mycoplasmas and prokaryotes, without cell wall have been placed under the class mollicutes (Latin mollis = soft, pliable + cutis = stain) and the order Mycoplasmatales. Mycoplasmas or mollicutes (soft skin) are without cell wall and are 'bounded by triple layered membrane.
- They are smallest microorganism which have been known to cause a number of diseases in animals and human being. Louis Pasteur first noticed them while observing the causative agent of pleuropneumonia in culture. He was unable to isolate them in a pure culture medium.
- **Nocard and Roux (1898) of Pasteur's laboratory cultured the microorganism in media containing serum and demonstrated that the pleomorphic microbes could produce the disease in inoculated healthy cattle. These were pleomorphic and were called PPLO (Pleuropneumonia like organism).**

This organism was later on given the name *Asterococcus mycoides* by Borrel *et al (1910).*

Characters of Mycoplasmas and Viruses

Properties	Virus	Mycoplasma
1. Growth on culture medium	-	+
2. Cell wall/cell wall Peptidoglycan lack	+	+
3. Generate metabolic energy	-	+
4. Depends on host cell nucleic acid for multiplication	+	-
5. Can synthesize protein by own enzyme	-	+
6. Require sterols	-	+
7. Visible in optical microscope x 1500	-	+
8. Filterable through 450 nm filters	+	+
9. Contains both RNA and DNA	-	+
10. Growth inhibited by antibody alone	-	+
11. Growth inhibited by antibiotics	-	+
12. Action on protein synthesis + positive action, negative action	-	+

(1) They are very small, non-motile (except *Spiroplasma)* and prokaryotic which may be parasitic or saprophytic.
(2) They lack cell wall and the outer boundary of the cells being the cytoplasmic membrane which is three layered unit membrane structure.
(3) Their cells possess plasticity (pleomorphic) and can assutp.e various shapes ranging from spheres to branched filaments.
(4) The plasticity allows the cells to pass through bacteriological filters even though the bmallest cells are about 0.3 nm in diameter.
(5) They require sterols for their growth. They are sensitive to supersonic vibrations desiccations and most of the physical environment factors.
(6) The colony of mycoplasma on solid agar medium appears as just like fried egg under stereo microscope. The colony shows spherical or hemispherical portion in the centre, which is surrounded by stlrface growth towards periphery. The typical colony is biphasic with a fried egg appearance (characterized by opaque, granular central area with a transleucent peripheral zone).
(7) They are susceptible to lysis by osmotic shocks caused by sudden dilution of the medium with water.
(8) They can be cultivated *in vitro* on non-living media of rich composition as facultative anaerobes or obligate anaerobes.
(9) The genome size of mycoplasmas are about 1/5 to 1/2 the size of those of bacteria.
(10) Genetic material is naked circular chromosome of fibrillar (double stranded) DNA, about 3 nm thick with a molecular weight ranges from 44 x 10 to 1200 x 10 daltons. Guanine: cytosine ratio of DNA ranges from 24-10%.
(11) The mode of multiplication is presumed to be by budding or binary fission.
(12) Chemically they are much closer to bacteria because they possess 4% DNA and 8% RNA, 70s ribosomes are present in the cytoplasm.
(13) Cells are non-motile but gliding motility has been observed in a few species.
(14) Mesosomes are not found in the cells of mycoplasma, but plasm ids are found on the basis of dry matter 50-80% proteins, 8-17% RNA and 4-7% DNA is present.

(15) They do not show response towards Gram staining i.e. they are Gram negative in nature**. Stevens & Fox** suggested a rapid and simple technique called **Dien's stain for staining technique.**
(16) They are insensitive to penicillin, vancomycin and cephaloridine (which effect on cell wall) but are sensitive toward tetracycline & chloremphenicol (which effect on metabolic activities) and they have harmful effect on them.

CLASSIFICATION

Class - Mollicutes
Order - Mycoplasmatales
Family - includes 3 families
(i) Mycoplasmataceae - e.g. *Mycoplasma*
(ii) Acholeplasmataceae - e.g. *AcllOleplasma*
(iii) Spiroplasmataceae - e.g. *Spiroplasma*

CELL STRUCTURE

- The ultra structure of cell of *Mycoplasma* appears as prokaryotic unicellular microorganism. The outer most boundary of cell is a unit plasma membrane which is three layered made up of lipoprotein. The chemical nature of lipoprotein consist of phospholipids and cholesterol. This unit membrane is 80-100 Å in thickness and selectively permeable. The membrane surrounds the cytoplasm which is packed with 70s ribosomes, R.N.A., naked circular chromosome of fibrillar double stranded DNA of about 3 nm thick, one or more electron dense areas and some empty vacuoles.
- Mesosomes are not found in the cells of *Mycoplasma* but plastnids are found 40 different types of enzymes are found in cytoplasm.
- Because the *Mycoplasma* are able to pass through many filters and grow in media which do not contain living tissue. They are therefore considered to be microorganism intermediate between bacteria and viruses chemically they are much closer to bacteria.

Reproduction

Formation of elementary body is an important mode of reproduction in *Mycoplasma.* The cells of *Mycoplasma laidlawii* show unequal division at the time of multiplication, as a result of which elementary bodies of 330-450 μm size are formed. They are very minute and can live freely. They are known as primary bodies. These primary bodies increases in size and shape accordingly called as secondary and tertiary structures. Inside these larger bodies the elementary bodies are formed and this stage is known as quarternary structure and after the rupturing of larger bodies these are released and this quarternary structure develops into complete mycoplasma cell.

ECONOMIC IMPORTANCE OF MYCOPLASMA

Mycoplasma causes many diseases in plants animals and human beings. The important ones, caused in plants are as follows:

Mycoplasmal Plant Diseases :

- Little leaf disease of Brinjal.
- Bunchy top of Papaya.
- Witches broom of Legumes.
- Yellow dwarf of Tobacco.
- Sandal spike disease.
- Sesamum Phyllody.
- Stripe disease of Sugarcane.
- Witches broom of Potato
- Clover virescence.
- Clover phyllody disease.
- Cotton virescence.

Symptoms of Mycoplasmal Plant Diseases

(i) Plant associated with mycoplasma are infected in the sieve tubes of the plants causes upsetting the hormonal balance resulting in witchs broom growth (all the axillary buds grows and convert into bunch).
(ii) In some cases flower leaf assume the shape of foliage leaves (phyllody) and various intermediate stage between flowers and leafy sprouts can be found (antholysis). In many cases no anthocyanin are formed in petal.

(iii) Degenerative processes in the sieve tubes of infected plant causes plant stunting, wilting or leaf yellowing and reduction of leaf size.
(iv) Colour breaking in the calyx portion of infected flower causes greening of all flower parts (virescence) and transferred into green leaf like structure (Phyllody).
(v) Stem become flat in infected plant.
(vi) Excessive callose formation and cell necrosis occurs in the sieve tubes of infected plant which can be visible under a fluorescence microscope after staining with aniline blue (serves as indirect indication for the presence of mycoplasma).

Transmission of Disease
(i) Plant mycoplasmas are known to be transmitted by certain insect vectors (leaf hopper) of cicadellidae and psyllidae. Once mycoplasma invade the insects salivary glands the vectors can transmit these organisms with the help of their saliva to healthy plants.
(ii) By grafting, mycoplasma can be transferred to healthy plant.
(iii) With the aid of *Cuscuta,* mycoplasma can be transferred from diseased to healthy plants. (with the help of haustoria) but this type of. transmission does not occur in nature.
(iv) An aphid named *Acnjthosiphon pisum* transmit mycoplasma in *Pisum sativum.*

Mycoplasmal Human Diseases
(i) *Mycoplasma pneumoniae* causes the disease primary atypical pneumonia (PAP) in the mouth, pharynx and genitourinary tract.
(ii) *Ureaplasma ueralyticum* have been found in women experiencing repeated or habitual reproduction failure.
(iii) Two species of mycoplasma viz. M. *hominis* and M. *fermentants* has been found responsible for infertility in men.
(iv) M. *orale* and M. *salivarium* are found responsible for respiratory tract infection.
(v) Mycoplasma have also been found in cases of arthritis and inflammation of the middle ear.

Spiroplasma are spiral form mycoplasmas. The helical filamentous forms are motile and show rapid rotary or screw motion and slow undulation motion. They are gram positive.

Mycoplasma resembles L-form in
(i)having similar ultrastructure
(ii) soft pleomorphic cells devoid of mucopeptide wall
(iii) not osmotically fragile
(iv) growth on media without osmotic protection.

They differ from L-forms in the following characters :
(i)while the L-forms revert to normal cells when the antibiotic is removed, mycoplasma never synthesizes the wall and
(ii) while L-forms are non-pathogenic, mycoplasmas are important pathogens.

L- form was isolated by Kleinberger Nobel in 1935. The cells were called L- forms after Lister Institute in London where they were isolated. L-forms are spheroplast like structure lacking cell wall. These naked protoplast can also be isolated from *Salmonella,E. coli* and *Proteus* (both Gram positive and Gram negative) as well as from other bacteria by cultivation on serum agar with penicillin (100 μg/ ml) in laboratory conditions. They produce fried egg type colonies which resemble those of mycoplasma species.

Two types of L-forms have been isolated. unstable L-forms, spheroplasts that are capable of dividing, but can revert to the original morphology, and stable L-forms, L-forms that are unable to revert to the original bacteria

The L-form colonies can be lifted and cultured at higher concentration of penicillin as at lower concentrations the L-forms revert to normal bacterial cells with walls. L-forms resemble protoplasts and sphaeroplasts in
(a) lack of flagella.
(b) inability to sporulate
(c) lack of some or all cell wall antigens
(d) revers on to normal cells when antibiotic treatment is stopped.

Fungi

- heterotrophs ⇒ First digest then absorb by haustre or Directly from living cell.
- Non motile reproductive structure
- filamentous body → mycelium → hyphae → Septum
  - heterokaryonic
  - Homokaryonic
  -] Dikaryotic
- chitin cell wall
- Nuclear mitosis = due to lack of centriole

Septum → porous structure that allow ribosome Mito, even Nucleus → so functionally Coenocytic condition

if absent → Coenocytic hyphae

3 kinds of reproductive structure

- Sporangia → Spores
  - – Non motile
  - – asexual or sexual
- gametangia
- Conidiospore

The oyster mushroom, Pleurotus ostreatus, not only decomposes wood but also immobilizes nematodes

Heterothalic

↳ fertilization b/w 2 genetically distinct gametes (from different thallus)

homothalic

↳ Both gametes are formed on same thallus

(1) Vegetative reproduction

- fragmentation
- Budding
- Fission

] in non-mycelic form

(2) Asexual reproduction by Spores

→ formed by Mitosis

(A) Sporangiospores → at tip of filament

- Zoospore → flagellated, Motile
- Aplenospore → Non flagellated, Non motile

(B) Conidia → → germinate → Mycelium

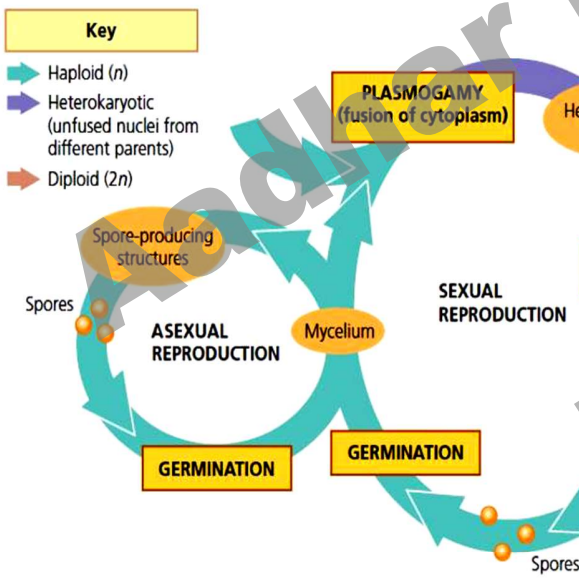
(C) Chlamydospore = in Adverse conditions

(D) Oidia = in favorable conditions . fungal cells separated and become spores

Sexual reproduction

3 steps ← Plasmogamy (Dikaryotization)
Karyogamy (Synkaryon formation)
Meiosis

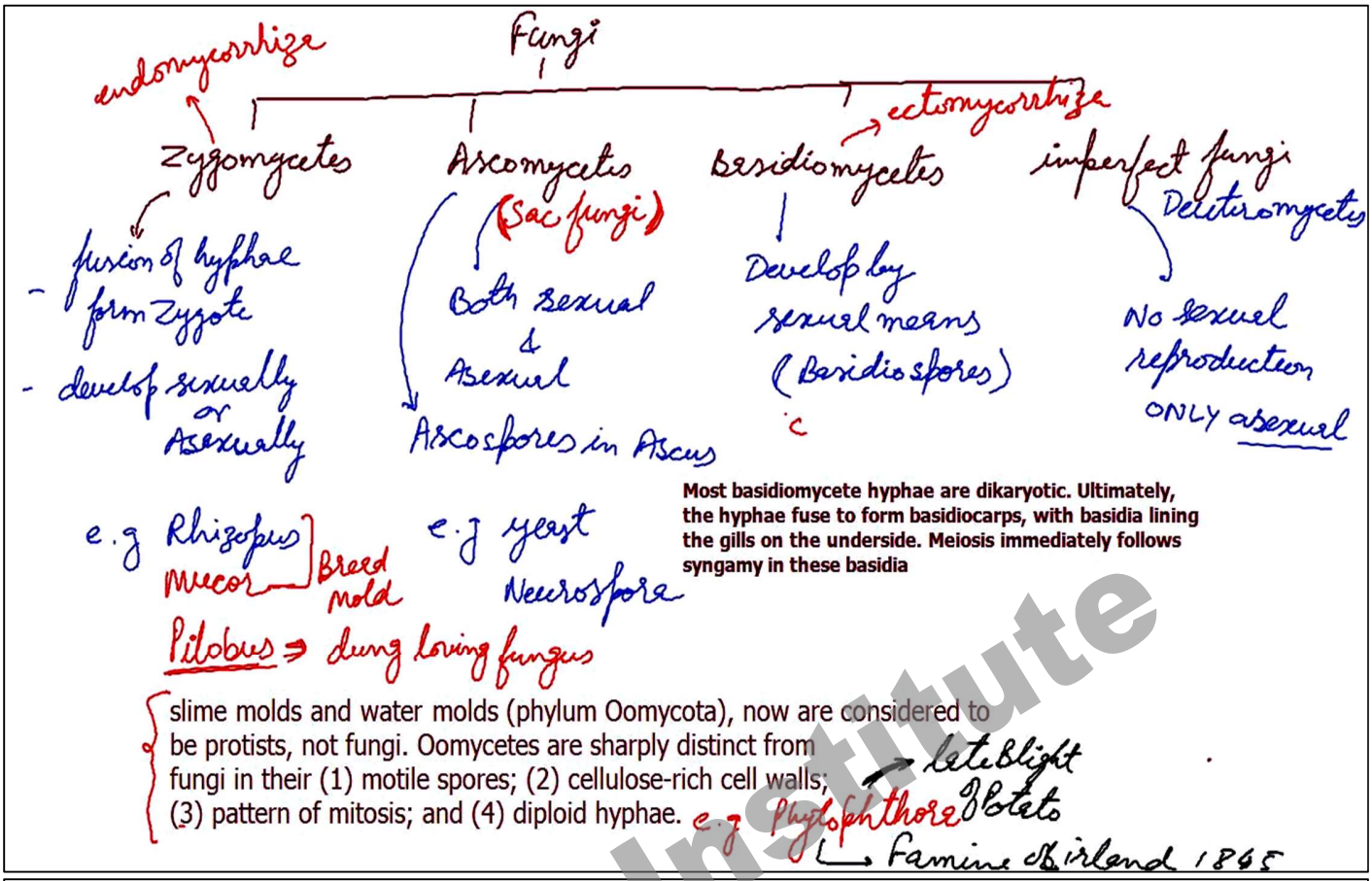
Various types → (A) Planogametic → any vegetative cell start acting as sex cell (gametangia)
↓ mitosis
gamete → become flagellated → fusion → meiosis → zoospore → mycelia

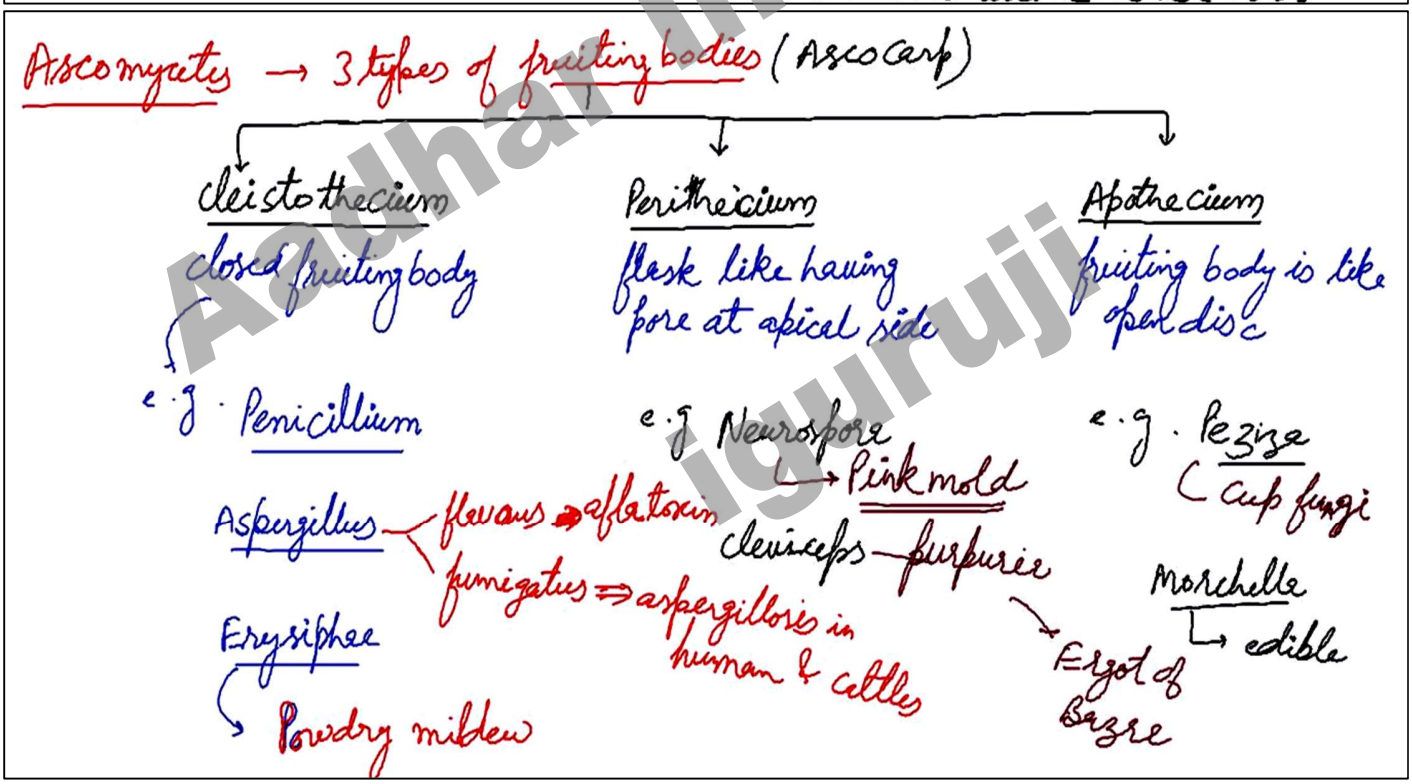


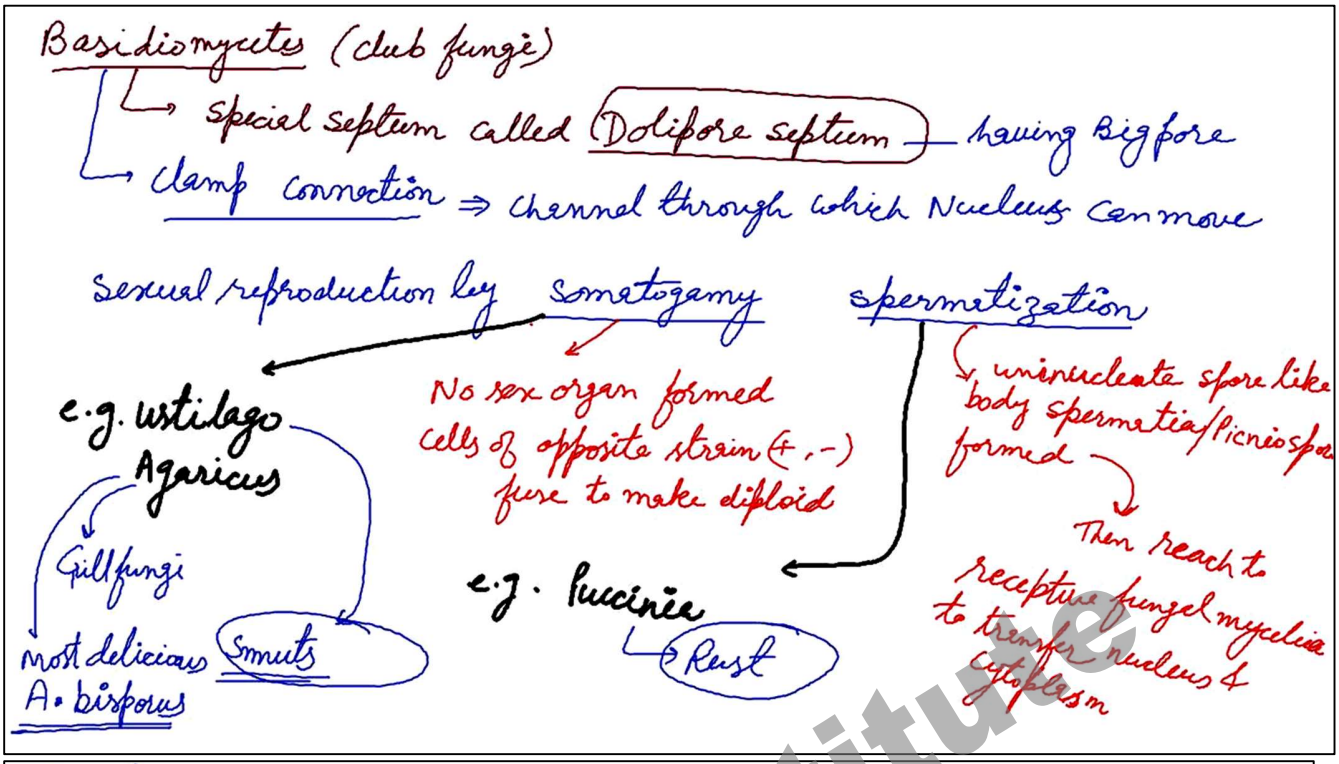
(B) Gametangial contact

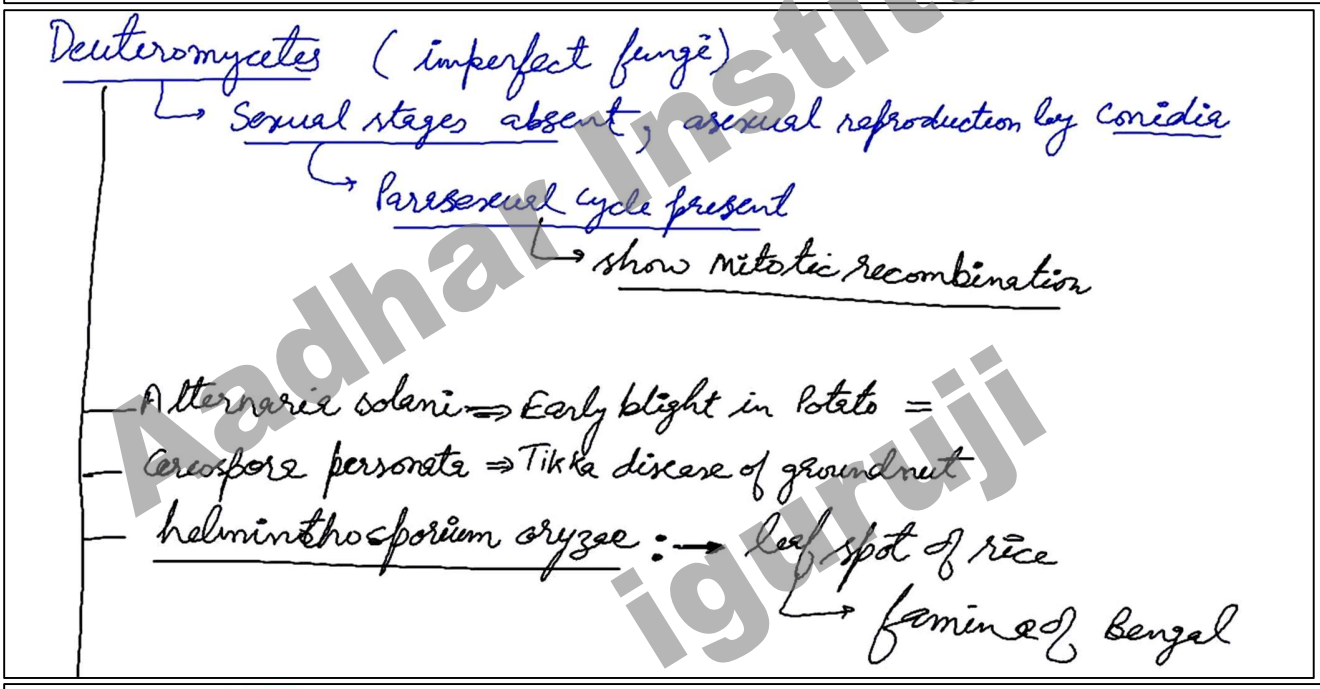
Mycelium ♂ → Antheridium
Mycelium ♀ → Oogonium
Antheridium → fertilization tube → fusion → Oospore → Meiosis → Sporangia → zoospore → mycelia
Oogonium → fusion

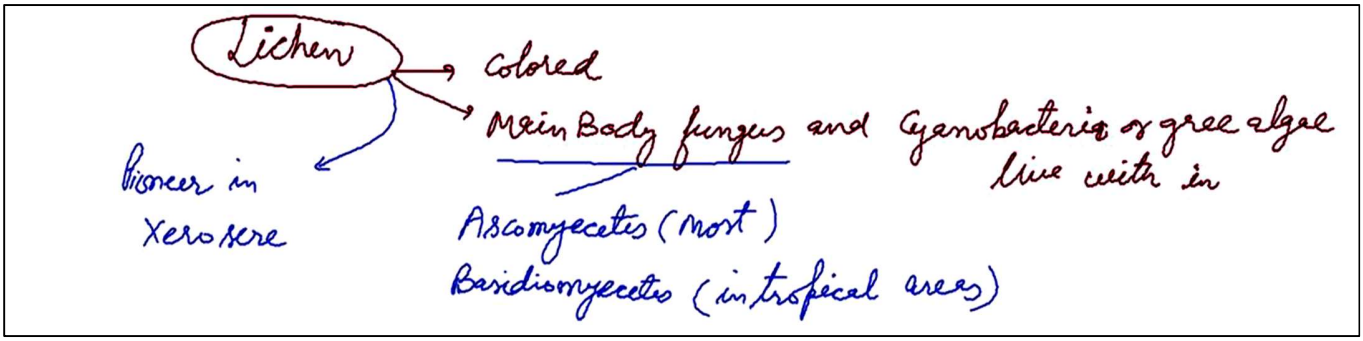
(C) Gametangial copulation ⇒ No fertilization tube formed and gametangia fuse to form zygospore → meiosis → formation of germinal tube → swollen part Sporangia → all haploid Nucleus migrate and make spores

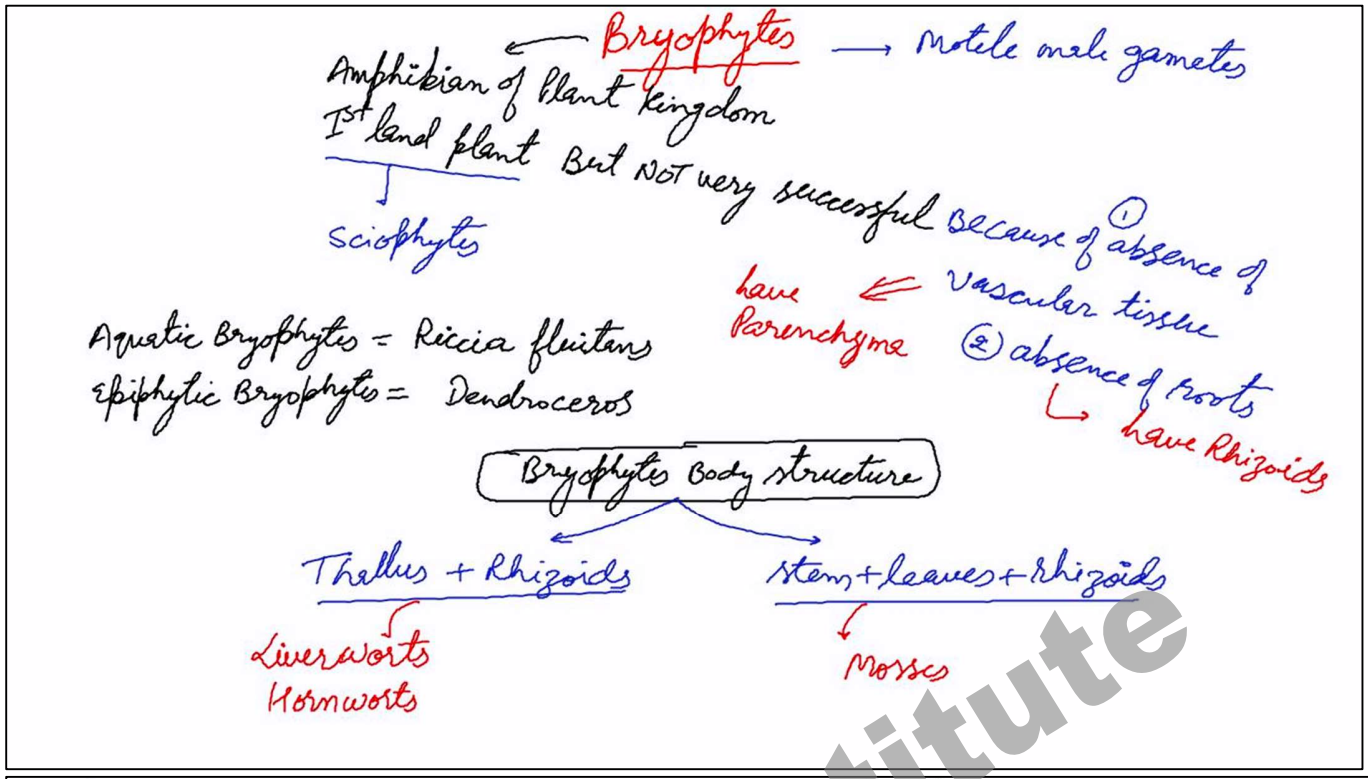


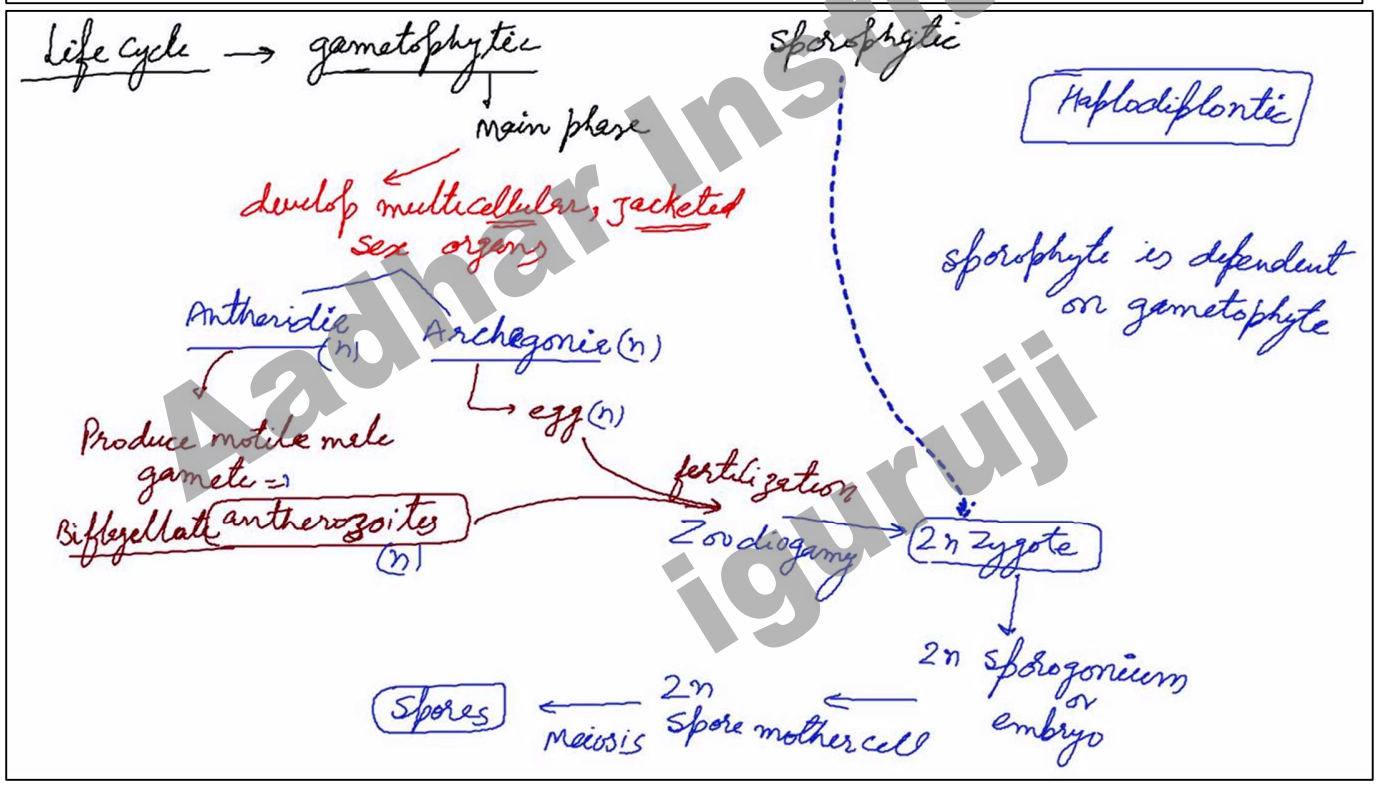










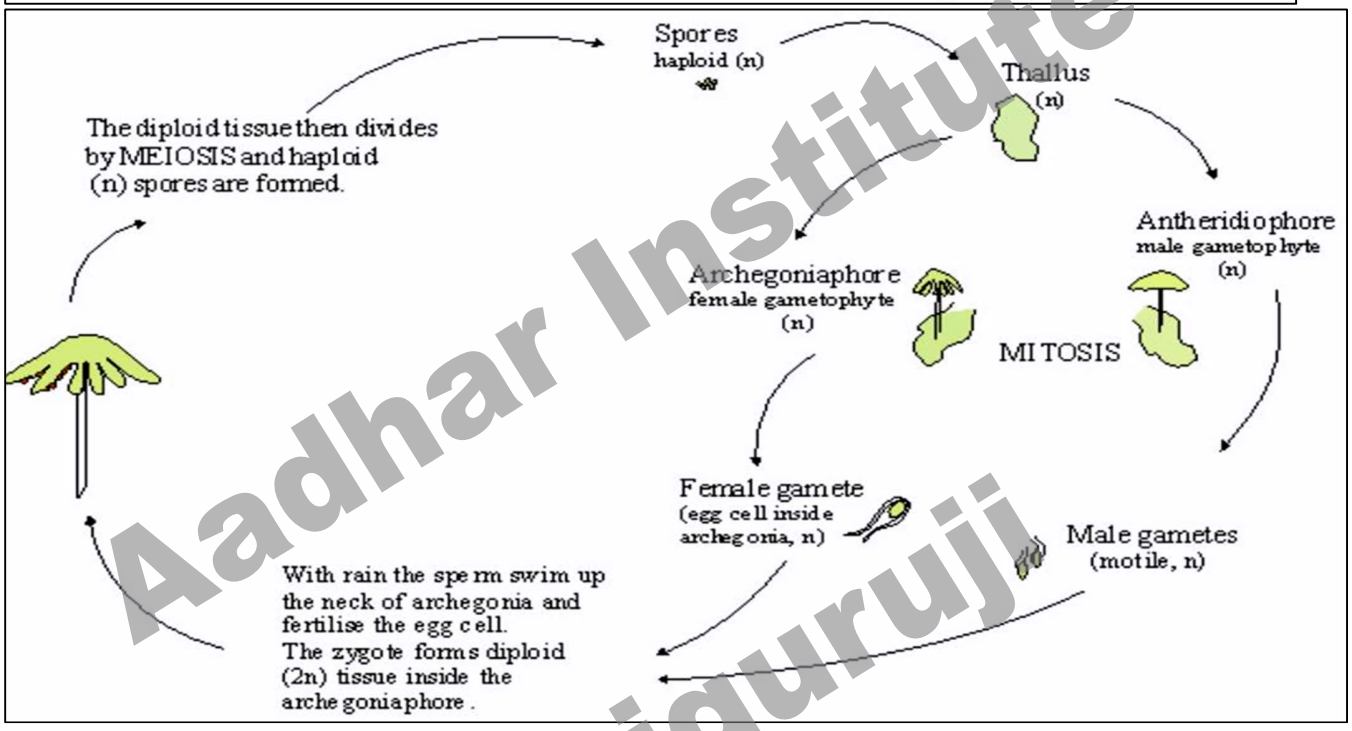


Spore germination

in Liverworts & Hornworts → direct → gametophytic Body

in Mosses (gregarious) → appear in groups

Protonemal → Buds → gametophytic body } indirect



Bryophytes

Hepaticopside (liverworts)

- Thallus + Rhizoids
- Sporophyte are completely parasite on gametophyte
- made up of Capsule (foot & seta absent)

e.g. Riccia
Marchantia
cryptothallus

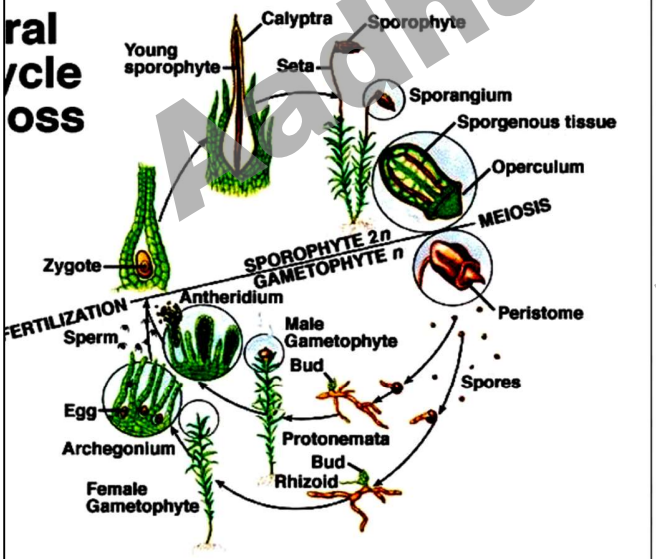
Anthoceropside (Hornworts)

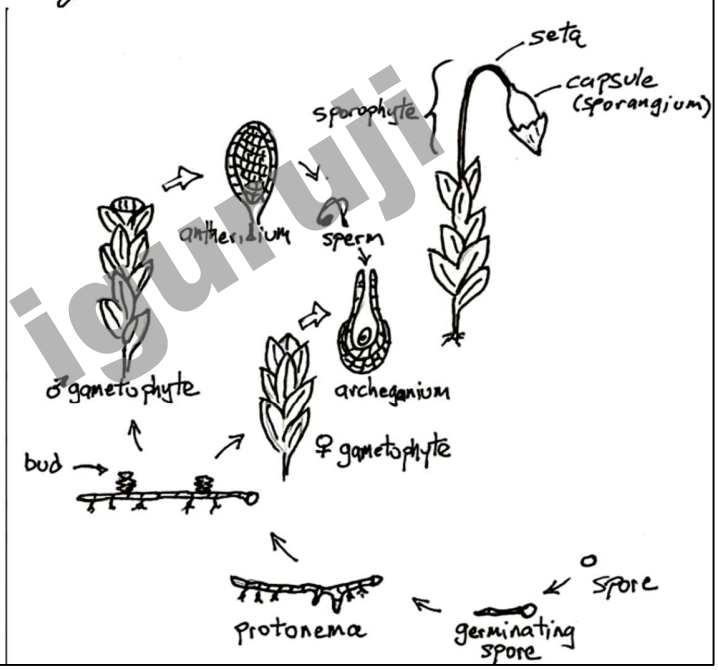
- Thallus + Rhizoids (Scales absent) (unicellular)
- Sporophyte are (2n) Semiparasite on (n) → for water
- Photosynthetic
- * have meristem at Basal part
- e.g. * Anthoceros ⇒ have algae like Characters
- Primitiveness

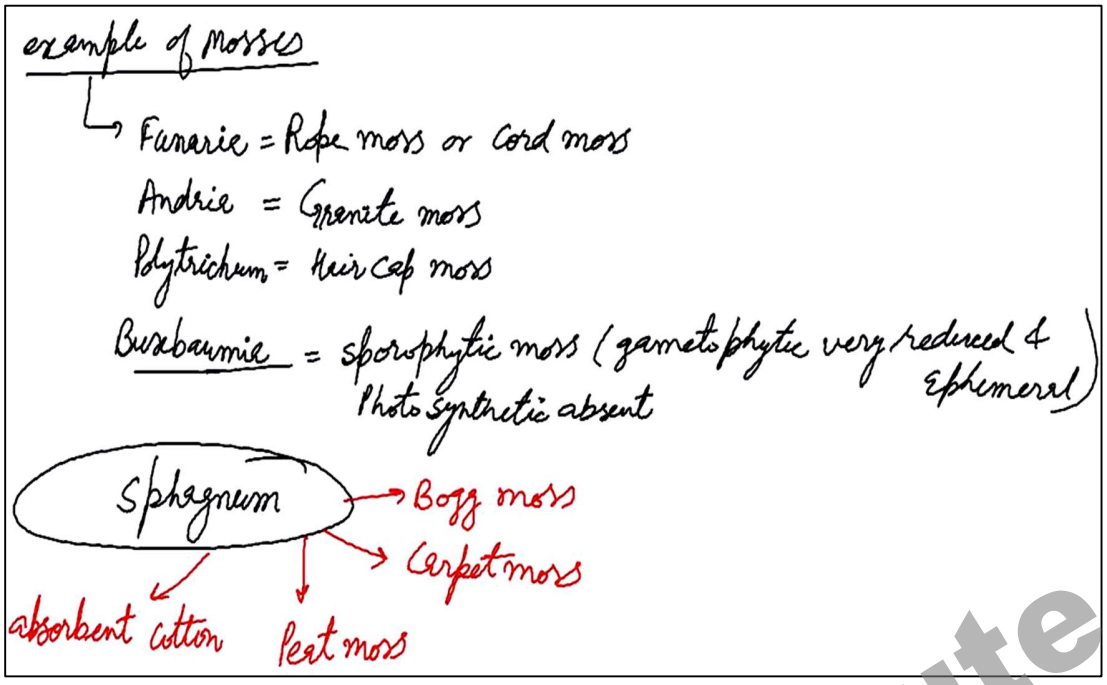
Bryopside (Musci) (Mosses)

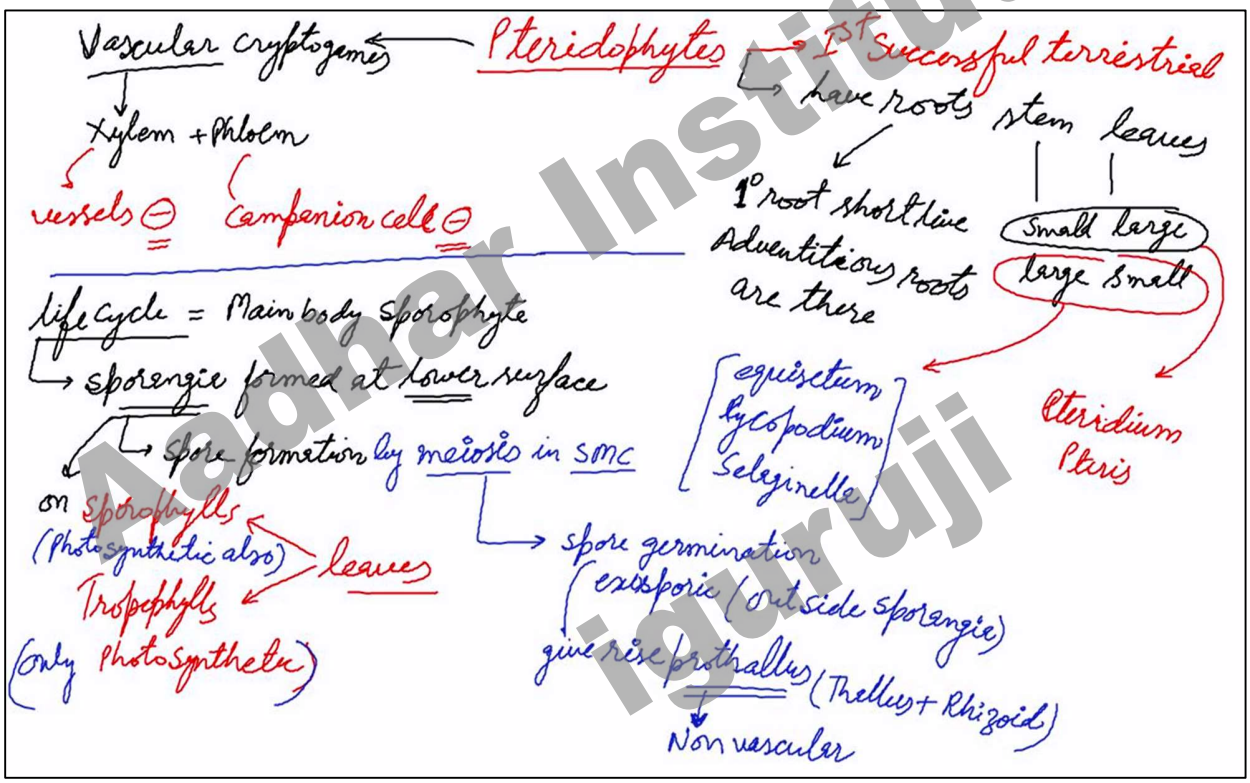
- Stem + leaves + Rhizoids
- ↓ uniqueness
- Multicellular Branched oblique septa
- Sporophyte highly develop
- foot → water absorption from gametophyte
- seta
- capsule → fertile

Seta → remains in the air due to which the dispersal of spores can takes place conveniently









- **Arabidopsis seeds have certain requirements for germination, including a period of dormancy (which can be substituted for by cold treatment) and light (a phytochrome response).** Mutations in a gene called ***DAG1* (*Dof Affecting Germination1*)** cause seeds that germinate in the dark without a dormancy period. Dof proteins are zinc finger transcription factors. The gene is expressed in the maternal tissues and all seeds of a mutant show this phenotype even if they result in pollination by a wild type (i.e. the embryo is wild type). Therefore, the maternal tissues during seed development control the dormancy behavior of the seed after being shed from the plant.
- **Upon imbibition, active metabolism resumes**. Imbibed seeds contain high levels of GA. It is produced by the germinating embryo and stimulates the synthesis of hydrolytic enzymes by inducing the transcription of their genes. These enzymes appear after radicle elongation and are therefore postgerminative. The hydrolytic enzymes include proteases, amylases and lipases that break down storage compounds making building blocks available to the growing seedling. One enzyme of particular importance is a-amylase which cleaves starch into glucose and maltose molecules. This reaction is of economic importance to the malting industry and so the regulation of a-amylase gene expression has been carefully studied. It is transcriptionally induced by GA. Plants also contain a unique metabolic pathway called the glyoxylate cycle. This enables plants to convert fatty acids of the stored lipids into carbohydrates, specifically glucose and sucrose. In contrast, animals are unable to convert fatty acids to glucose.
- **GA and ABA act antagonistically to regulate the germination vs. maturation programs**. ABA promotes maturation while GA promotes germination. As mentioned, ABA is necessary for seed maturation because ABA deficient mutants are viviparous and desiccation intolerant. Therefore, without ABA, seeds directly enter the germination program. Exogenous ABA can inhibit germination following dormancy. Conversely, promotes germination. GA is required for germination because GA deficient mutants are unable to germinate. Exogenous GA application to developing seeds can block maturation and induce vivipary.
- The VP1/ABI3 protein is a central regulator in these functions. This protein is a transcription factor that promotes the expression of maturation genes and inhibits the expression of germination genes. Mutants in this gene are ABA insensitive.

Seed dormancy

The presence of dormancy in a seed is the most important characteristic feature. Because of the this character, seeds remains viable for many years. The seeds are dispersed very far places through water, air or insects. Most of the seeds are unable to germinate just after dispersal but germinates after sometime. The time between the maturation and germination of seed is known as "Dormancy period". The state of inhibited germination as a result of internal causes is usually called 'dormancy'. This seed dormancy is of considerable advantage to the plant which help in adverse environmental conditions. The embryo remains inactive in this period and all the growth processes suspended temporarly.

There are three main basic reasons for dormancy of seed:

1. Impermeability of seed coat: The seed coats of many species of ***leguminoseae*** **and** ***convolulaceae*** families are completely impermeable to moisture [water] and oxygen at the time of their maturity. These seed coats of those seed are thick and hard. Their cell wall is covered by a layer of lignin which is water proof coating.

Such seed are taken more time for germination. The dormancy of seeds breaks by different artificial methods:

(i) By making minute hole/pores with help of pointed sharp apparatus on the seed coat.

{ii) The seed are rubbed on hard object so that seed coat become thin.

(iii) The partial degeneration of seed coat is carried by the action of sulphuric acid. Under natural conditions seed dormancy is gradually over come by the action of microbe [bacteria] in soil, in the alimentary canal of fruit eater birds and due the presence of high cold or high temperature.

2. Dormant Embryo: In many species, although, the embryo completely not matured when the seed is ripe, even than it fails to germinates when ever the environmental conditions are favourable or even seed coat is removed. This is known as "Embryo" dormancy". Normally embryo dormancy occurs in many plants and fruits yeilding plants of forest. This condition is achieved due to physiological action of seed. Such type of seed must complete their enzymatic and chemical reaction before the germination of seed. In the lack of these reactions, seed are unable to germinate, such

seed are kept in low temperature! and in desirable moisture. Due to this seed dormancy can be broken. The low temperature is the main reason for germination of seeds of Apple, peaches, pears, mapple and pine etc.

3. Germination Inhibitors: Germination of some seeds is sometimes checked or prevented by the presence of some chemical compounds are called "Germination Inhibitors" such as Ferulic acid present in tomato juice, Caumarin. Abscissic Acid, Dormin and para ascorbic acid etc. This condition is generally found in xerophytic plants. These germination inhibitors washed away with water is known as "Leaching".

Method of Breaking of seed Dormancy
(i) Scarification: The hard seed coat is broken in this method so that water and oxygen enter into the seed coat.
(ii) Stratification: The seed kept at low temperature and in the presence of oxygen and water for some time, so that embryo can completes its germination period,
(iii) Light Requirement: The plants which is affected by the light are known as "Photoblastic seeds". The seeds which germinate in the presence of light are called "positive photoblastic seeds", such as Lattuce, Capsella, Lepidium and Tobacco etc. The seeds in which germination take place in the absence of light are called negative photoblastic seeds, such as *Nigella* and *Silene* etc. Some seeds are not affected by light are called nonphotoblastic seeds.

The dormancy of photoblastic seeds can be broken by the treatment of **red light.** The phytochrome red, absorbs red light and convert into phytochrome far red which increase the germination of seed.
seed + R [red light] —» Germination
seed + R + FR —> No germination
seed + R + FR + R ----» Germination
Seed + R + FR + R + FR -----» No germination

(iv) Alternative Temperature: The treated with high and low temperature alternatively. It increases the germination of seed. e.g.The seed of sweet clover and Alfa - alfa are treated at 2000 atmospheric pressure and 18°C temperature.
(v) Viability: This is called the existence of life in a seed. The viability of seed can be find out by 2, 3, 5. triphenyle tetrazolium chloride. The embryonal axis of living seed becomes pink in colour in the solution of T.T.C.
(vi) Seed dormancy also broken by the treatment of G.A.

SPECIAL POINTS
(1) Hard and impermeable seed coat is found in most of **legume plants** which is the main reason of seed dormancy.
(2) Growth inhibitor is present in the endosperm of **Iris.**
(3) If a fleshy sweet part of fruit is present in one seed and a fleshy bitter part of fruit is present with another seed than both seeds are unable to germinate.
(4) The seed dormancy in **Eranthis** heemalis and **Ginkgo** is present due to immature embryo.
(5) A hard lignin water resistant layer [seed coat] is present on seed of some **Leguminosae** plants,
(6) Growth inhibitors are present in seed coat of cucurbita and in the embryo of **Xanthium.**
(7) Strong mineral acids have been used successfully to interrupt seed dormancy caused by resistant or hard impermeable seed coat, such as **potassium nitrate, Ethylene, Chlorohydrine** and **Thiourea** etc.

Xenia (by Focke): Effect of pollens on structure inside Embryosac except Embryo → on Endosperm character — Maize.
Metaxenia: Effect of pollen on structure out side the Embryosac or Endosperm — Date palm - maturity time as well as size of fruits can be changed by using different pollens.

The buds are of two types:
(1) Vegetative bud (2) floral bud, before showing their respective growth, the bud of many plants undergo a dormant phase. This period starts in late summer and terminate in the spring season.

Causes of Bud Dormancy:

- According to Hemberg in woody plant the bud dormancy is caused by Abscissic acid. The level of endogenous ABA increases with the onset of dormant period and decreases when it is broken. In non woody plant like potato, the bud dormancy is again due to an inhibitor. Bannet Clark and Kefford 1953 identified this substance as **Inhibitor-*beta*. It present in the peel of dormant potato tuber.**
- **The inhibitor-*beta* is responsible for checking the sprouting of buds located in the 'eyes' of potato tuber,**

Perception of Dormancy by Buds: According to wareing. The bud dormancy, at least in woody plant, is caused by long day length, while it is broken by short day. **Breaking of Bud Dormancy**

(i) Chilling: The bud dormancy of some plant can be broken, if they are given cold temperature treatment for specific duration,

(ii) Alternating temperature treatment: The dormancy of buds of some plants can be broken, if they are subjected to low temperature (0°-10°C) for a brief duration and then given warm treatment.

(iii) High temperature treatment: If dry potato tubers are stored at 35°C or moist at 20°C, the I dormancy of tubers buds is broken.

(iv) Chemicals: Some chemicals like 2-chloro ethanol, gibberellin and thiourea are capable of breaking of dormancy of buds. Of these 2-chloro ehtanol is very effective in breaking the dormancy of potato tube buds. Endogenous gibberellin play a very significant role in controlling the dormancy of potato tuber.

(v) Gene Derepression: The genome of the dormant potato bud lacks the ability of DNA dependent RNA synthesis. By treating the bud with ethyl chlorohydrin or gibberellin, the ability of RNA synthesis is achieved, Thus causing derepression of a repressed gene.

Development of fruit

(1) Development of fruit

Fruit is defined as the **ripened ovary**. Normally, a fruit develops from the ovary after fertilization *(true fruit).* The ovary wall undergoes the ripening changes and forms the fruit wall, called the **pericarp**. The ovules develop into seeds.

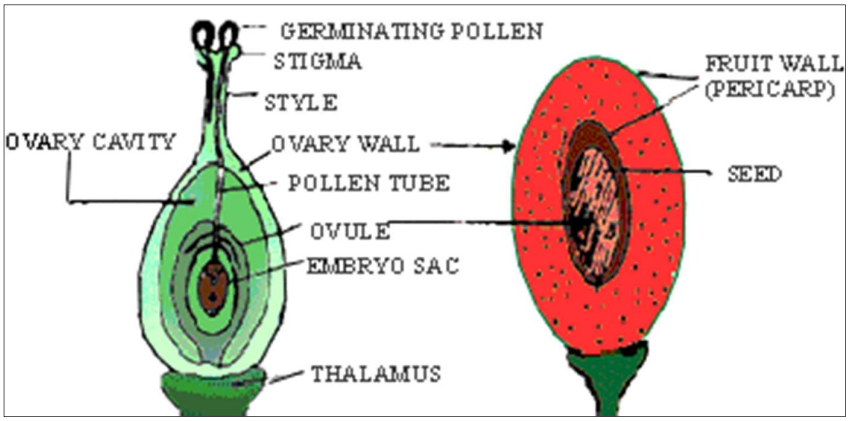


Figure 15.27 Development of fruit (diagrammatic)

- In many fruits, along with the ovary, certain other parts of flower or inflorescence (e.g. thalamus, calyx, inflorescence axis, etc) also participate in fruit formation (*false fruit*).
- During ripening (maturation), various structural, physiological and biochemical changes take place in the fruit wall (ovary wall). Fertilization provides the stimulus necessary for initiating these changes. Some plant hormones are probably released in the ovary by the pollen tube during fertilization. Also, the developing seeds presumably release hormones enhancing the ripening changes. Auxins, gibberellins and kinins are believed to play an important role in the process.
- The mature fruit may have one or more seeds. The pericarp at maturity may remain soft and fleshy or may become dry. It may be dehiscent or indehiscent.
- Since flowers with closed ovaries are present only in angiosperms, fruit is formed only in angiosperms (flowering plants).

Contributions of different flower parts to the fruit

- Most fruit develops from the ovary. In fact some schemes classify fruit derived from a single ovary as “true fruits” while “false fruits” are composed of tissues derived from flower parts other than the ovary or from more than one ovary.
- In “true fruits” the outside of the fruit is called the pericarp and develops from the ovary wall. The pericarp can be dry and papery, like in maple or dandelions, woody like in nuts or fleshy as in berries (grapes and tomatoes) and stone fruits (cherries and peaches). These pericarp differences reflect adaptations to different dispersal mechanisms (eg. wind for papery pericarps, animal consumption for fleshy fruits). The fruit can contain a single seed as in corn, or many seeds like a pea pod or pumpkin. The pericarp of some fruits is further differentiated into specialized layers called exocarp, meso- and endocarp. For example in citrus the rind is the exocarp, the white covering is the mesocarp and the juice sacs are the endocarp.

- Many fruits we consider berries, such as raspberries and strawberries, are botanically not classified as berries. Raspberries are examples of aggregate fruits. Each juicy little sphere is actually an individual fruit of the same class as cherries, and what we consider as the fruit is really an aggregation of fruits.
- Strawberries and apples are examples of accessory fruits, where some of the fleshy tissue is derived from flower parts other than the ovary. Strawberry fruits are actually what we consider the seeds. They are called achenes, which are dry fruits in the same category as dandelions. The fleshy part that we eat develops from the receptacle. Most of the fleshy tissue in apples develops from the hypanthium which is a region of the flower where sepals, petals and stamens are all fused to the ovary. Thus all floral organs contribute to the fleshy portion of apples.

Phases of fruit development

- Fruit development can generally be considered to occur in four phases: fruit set, a period of rapid cell division, a cell expansion phase, and ripening/maturation.
- **Fruit set** involves the decision whether to abort the ovary or proceed with fruit development. Fruit set is normally dependent on pollination. Pollen triggers fruit development indicating that positive signals are generated during pollination. In the absence of these signals, the flowers abscise. Growing pollen produces GA and application of GA can induce parthenocarpic fruit, therefore it is believed that GA is a triggering signal. Lagging slightly behind the growing pollen tube is a wave of increased auxin production by the style and then the ovary. Auxin application can also induce parthenocarpy and so it is thought that GA acts by inducing auxin production. However, most GA deficient mutants are able to produce fruit indicating that this is not the sole mechanism to induce fruit development and in an auxin insensitive tomato mutant, fruit growth is normal.
- Continued fruit development usually relies on the continued presence of developing seeds. Seed abortion or removal causes fruit abortion, which can be reversed with auxin application. For example. removal of strawberry “seeds” prevents the development of the receptacle as a “fruit” but if auxin is applied following seed removal, fruit development continues. Commercial crops that produce parthenocarpic (seedless) fruits, such as bananna, often show quantitaive or qualitative differences in GA or auxin content in the ovary when compared to nonparthenocarpic varieties.
- The **phase of rapid cell division** involves all growing parts of the fruit. This is thought to be controlled by the developing seeds. The number of fertilized ovules in a fruit is correlated with both the initial cell division rate and the final size of the fruit. Also, fruits with an uneven distribution of seeds are often lopsided. There is a correlation between cytokinin levels in developing embryos and cell division in surrounding tissues but there is no direct evidence that embryo cytokinin in fact regulates fruit cell division. It is difficult to reconcile the complete development of parthenocarpic fruit with the requirement of embryos for cell division except to say that parthenocarpy represents an abnormal situation.
- The cell division phase gradually shifts into the **cell expansion phase**. The rate and duration of cell division varies among fruits and also among tissues within a fruit. Tissues made up of many small cells at maturity continue dividing while tissues composed of large cells have begun expanding. In tomato the cell division phase lasts approximately 7-10 days while cell expansion lasts 6-7 weeks. Cell expansion accounts for the largest increase in fruit volume, often contributing in excess of a 100 fold size increase. Gibberellins are also associated with fruit expansion and removal of the seeds from pea pods inhibited GA biosynthesis in the pericarp. Many believe that auxins from seeds regulate cell expansion of the pericarp, but auxin application does not always compensate for seed removal, and in an auxin insensitive tomato mutant, fruit growth is normal.

Fruit ripening

- Ripening represents the shift from the protective function to dispersal function of the fruit. Ripening occurs synchronously with seed and embryo maturation, as described in the lecture on embryo development. In dry fruits (cereals, nuts, dandelions) ripening consists of desiccation and is considered maturation. Ripening in fleshy fruits is designed to make the fruit appealing to animals that eat the fruit as a means for seed dispersal. Ripening involves the softening, increased juiciness and sweetness, and color changes of the fruit. Fleshy fruits are either climacteric or non-climacteric. Climacteric fruits produce a respirative burst with a concomitant burst in ethylene synthesis, as the fruits ripen. These include fruits with high degrees of flesh softening, like tomato, banana, avacado, peach etc.
- Ripening has been most intensively studied in tomato. Ethylene is a major regulator of the ripening process. Inhibitioin of ethylene with inhibitors, transgenic approaches or mutants blocks ripening. Exogenous ethylene

accelerates ripening. There are also developmental factors involved because fruit does not attain competence to respond to ethylene until near the end of the cell expansion phase (the mature green stage). Several genes associated with ripening are ethylene inducible. This occurs transcriptionally in most genes but at least one is known where mRNA accumulation is regulated post-transcriptionally. None of these genes are induced until competence for ethylene response is attained.

- The tomato *never-ripe* mutation blocks fruit ripening and is insensitive to ethylene. The mutated gene is similar to the ethylene receptor isolated from arabidopsis, suggesting that *never-ripe*is an ethylene receptor mutant. *NR* mRNA is not expressed until the mature green stage, suggesting that lack of this ethylene receptor might be related to the lack of competence to respond to ethylene at earlier stages.
- Ethylene production is autocatalytic. That is, exposure to ethylene stimulates the synthesis of more ethylene. This occurs because the genes for the biosynthetic enzymes (e.g. ACC SYNTHASE) are ethylene inducible. The result is a positive feedback loop. Furthermore, the *Never-ripe* gene is ethylene inducible, resulting in a positive feedback loop for ethylene sensitivity as well. Both these factors contribute to the dramatic burst of ethylene production during ripening.
- Fruit softening involves a partial breakdown of cell walls. Several enzymes are known to be involved in this process. Polygalacturonase hydrolyzes bonds in pectins. The gene for this enzyme is ethylene inducible.
- Changes in fruit color involve changes in the expression of pigment biosynthetic genes. The major pigment in tomato is a carotenoid. The first committed step in carotenoid biosynthesis is catalyzed by phytoene synthase, and the gene for this enzyme is induced by ethylene.

Fate of the parts of the flower, floral organs

Ovule	**Seed**
Integuments	**Seed coats**
Nucellus	**Shrivels to thin papery layer**
Fusion nucleus of the embryo sac	**Endosperm tissue for food storage and nutrition of the embryo**
Ovum	**Embryonic plant**
Antipodals	**Usually shrivel, sometimes persists as absorbing mechanism for endosperm**
Synergids	**Usually shrivel and are absorbed by the developing embryo**

DEVELOPMENT OF THE PLANT EMBRYO in ARABIDOPSIS

- Plant embryos, unlike those of animals, do not contain organ primordia representative of the adult body-plan. The mature embryo of a flowering plant such as *Arabidopsis thaliana* comprises only two organ systems, the embryonic axis and the **cotyledons**
- The **embryonic axis** contains the basic layout of the seedling, and has a population of meristem cells at each end which give rise to adult structures of the plant during post-embryonic development. The axis is therefore organized into regions representing the **shoot apical meristem (SAM)**, the future seedling shoot **(epicoty1** and **hypocoty1)**, the embryonic root (radicle) and the **root apical meristem (RAM)**
- The cotyledons, which are attached to the hypocotyl, are storage organs that provide nourishment to the growing seeding. As well as being divided into organ-forming regions, the embryo is also organized into radial bands representing the three fundamental cell layers common to all flowering plants: **L1, L2** and **L3.**

➔three important aspects of embryonic development in flowering plants:

I.specification of the apical-basal axis,

II.patterning the axis to generate the organ-forming regions,

III.the control of cell differentiation.

During the course of pathogenesis, normal activities of the infected host plant undergo malfunction. Consequently, morphological and physiological changes occur.

A. Morphological or structural changes: Physiological malfunctioning of the host cells causes disturbances in chemical reaction which ultimately lead to some structural changes viz., overgrowth, phyllody, sterile flowers, hairy roots, witches broom, bunchy top, crown gall, root knot, leaf curling, rolling, puckering etc.

B. Physiological changes:

i. Disintegration of the tissues by the enzymes of the pathogen.

ii. Effect on the growth of the host plant due to growth regulators produced by

iii. the pathogen or by the host under the influence of the pathogen.

iv. Effect on uptake and translocation of water and nutrients.

v. Abnormality in respiration of the host tissues due to disturbed permeability of cell membrane and enzyme system associated with respiration.

vi. Impairing the phenomenon of photosynthesis due to loss of chlorophyll and destruction of leaf tissue. Effect on the process of translation and transcription.

vii. Overall reproduction system of the host.

Symptoms of Plant Diseases

A visible or detectable abnormality expressed on the plant as a result of disease or disorder is called *symptom*. The totality of symptoms is collectively called as *syndrome* while the pathogen or its parts or products seen on the affected parts of a host plant is called *sign*. Different types of disease symptoms are cited below:

Necrosis: It indicates the death of cells, tissues and organs resulting from infection by pathogen. Necrotic symptoms include spots, blights, burn, canker, streaks, stripes, damping-off, rot etc.

Wilt: Withering and drooping of a plant starting from some leaves to growing tip occurs suddenly or gradually. Wilting takes place due to blockage in the translocation system caused by the pathogen.

Die-back: Drying of plant organs such as stem or branches which starts from the tip and progresses gradually towards the main stem or trunk is called die-back or wither tip.

Mildew: White, grey or brown coloured superficial growth of the pathogen on the host surface is called mildew.

Rusts: Numerous small pustules growing out through host epidermis which gives rusty (rust formation on iron) appearance of the affected parts.

Smuts: Charcoal-like and black or purplish-black dust like masses developed on the affected plant parts, mostly on floral organs and inflorescens are called smut.

Blotch: A large area of discolouration of a leaf, fruit etc. giving a blotchy appearance.

White blisters: Numerous white coloured blister-like ruptures are surfaced on the host epidermis that forms powdery masses of spores of fungi. They are called white blisters or white rust.

Colour change: It denotes conversion of green pigment of leaves into other colours mostly to yellow colour, in patches or covering the entire leaves. (i) *Etioliation*: Yellowing due to lack of light, (ii) *Chlorosis*: Yellowing due to infection viruses, bacteria, fungi, low temperature lack of iron etc. (iii) *Albino*: Lack of any pigment and turned into white or bleached (iv) *Chromosis*: Red, purple or orange pigmentation due to physiological orders etc.

Exudation: Such symptom is commonly found in bacterial diseases when masses of bacterial cells ooze out to the surface of affected plant parts and form some drops or smear, it is called exudation. This exudation forms a crust on the host surface after drying.

Overgrowth: Excessive growth of the plant parts due to infection by pathogens. Overgrowth takes place by two processes (i) *Hyperplasia*: abnormal increase in size due to excessively more cell division (ii) *Hypertrophy*: abnormal increase in size or shape due to excessive enlargement of the size of cell of a particular tissue.

Atrophy: It is known as hypoplasia or dwarfing which is resulted from the inhibition of growth due to reduction in cell division or cell size.

Sclerotia: These are dark and hard structures of various shaped composed of dormant mycelia of some fungi. Sometimes, sclerotia are developed on the affected parts of the plant. Presence of sclerotia on the host surface is specifically called a sign of disease rather than symptom

Development of epidemics

Sudden outbreak of a disease within a relatively short period covering a large area and affecting many individuals in a population is called *epidemic*. Although, this term was originally designated to the human diseases, now applied in the diseases of animals, poultry, plants etc. Epidemic form of plant diseases is called as *epiphytotics*.

For a disease to occur, coincidence of three parameters of disease triangle is essential, namely, the vulnerable host, virulent pathogen and favourable environment. Under such circumstances, the pathogen not only completes its life cycle but also undergoes repeated generations. Then an epidemic develops only when few repeated generations are completed by the pathogen on the same host. As each generation or cycle of the pathogen takes a few days for completion, the fourth parameter i.e. time factor (forms a disease tetrahedron or disease pyramid) is also involved in epidemic build up. In other words, epidemic growth is both temporal (pertaining to time) and a spatial (relating to space or area) process. The initial stages of an epidemic growth curve have a lag phase, when the incubation period is longer, inoculum load is weak and prevalent environmental conditions are unfavourable. Subsequently, when the conducive conditions occur, the growth of the disease is rapid and the severity of the epidemic explodes like a time bomb. Later, severity declines either due to unfavourable weather or crop maturity or both.

Plant Disease Management

The word 'control' is a complete term where permanent 'control' of a disease is rarely achieved whereas, 'management' of a disease is a continuous process and is more practical in influencing adverse affect caused by a disease. Disease management requires a detail understanding of all aspects of crop production, economics, environmental, cultural, genetics and epidemiological information upon which the management decisions are made.

A. Principles of plant disease management: There is six basic concept or principles or objectives lying under plant disease management.

1. **Avoidance of the pathogen**: Occurrence of a disease can be avoided by planting/sowing a crop at times when, or in areas where, inoculum remain ineffective/inactive due to environmental conditions, or is rare or absent.
2. **Exclusion of the pathogen**: This can be achieved by preventing the inoculum from entering or establishing in a field or area when it does not exist. Legislative measures like quarantine regulations are needed to be strictly applied to prevent spread of a disease.
3. **Eradication of the pathogen**: It includes reducing, inactivating, eliminating or destroying inoculum at the source, either form a region or from an individual plant (rouging) in which it is already established.
4. **Protection of the host**: Host plants can be protected by creating a toxin barrier on the host surface by the application of chemicals.
5. **Disease resistance**: Preventing infection or reducing the effect of infection of the pathogen through the use of resistance host which is developed by genetic manipulation or by chemotherapy.
6. **Therapy**: Reducing severity of a disease in an infected individual.

The first five principles are prophylactic (preventive) procedure and the last one is curative.

B. Methods of plant disease management

1**. Avoidance of the pathogen:**
   i. Choice of geographical area
   ii. Selection of a field
   iii. Adjustment of time of sowing
   iv. Use of disease escaping varieties
   v. Use of pathogen-free seed and planting material
   vi. Modification of cultural practices
2. **Exclusion of inoculum of the pathogen**
   i. Treatment of seed and plating materials
   ii. Inspection and certification
   iii. Quarantine regulations
   iv. Eradication of insect vector
3. **Eradication of the pathogen**

i. Biological control of plant pathogens
ii. Eradication of alternate and collateral hosts
iii. Cultural methods:
  a. Crop rotation
  b. Sanitation of field by destroying/burning crop debris
  c. Removal and destruction of diseased plants or plant parts
  d. Rouging
iv. Heat and chemical treatment of diseased plants
v. Soil treatment: by use of chemicals, heat energy, flooding and fallowing

4. **Protection of the host**
   i. Chemical control: application of chemicals (fungicides, antibiotics) by seed treatment, dusting and spraying
   ii. Chemical control of insect vectors
   iii. Modifications of environment
   iv. Modification of host nutrition
5. **Disease resistance** Use of resistant varieties: Development of resistance in host is done by
   i. Selection and hybridization for disease resistance
   ii. Chemotherapy
   iii. Host nutrition
   iv. Genetic engineering, tissue culture
6. **Therapy** Therapy of diseased plants can be done by
   i. Chemotherapy
   ii. Heat therapy
   iii. Tree-surgery

Fungi

The term fungus (plural fungi) includes eukaryotic, spore-bearing, achlorophyllus, organisms that generally reproduce sexually and asexually, and whose usually filamentous, branched somatic structures are typically surrounded by cell walls containing chitin or cellulose, or both of these substances, together with many other complex organic molecules**General morphology, characters and somatic structures of fungi The thallus**: Thallus is a growth form lacking differentiation into root, stem and leaves. In fungi, it is known as somatic (soma= body) phase. It may be plasmodial, unicellular, pseudoplasmodial or mycelial. A filamentous structure of fungal body composed of multicells is known as mycelium (pl. mycelia) and a fragment (unit) of mycelium is called hypha (pl. hyphae i.e. web). Hyphae or mycelia may be septate (having cross wall in the filament) or aseptate (without cross wall or septum).

Branching habit of mycelium: Dichotomous, sympodial, lateral, opposite, verticilliate, monopodial etc.

Other somatic structures: Rhizoides (rootlike), appressorium (pl. appressoria), haustorium (pl. haustoria), hyphopodium (pl. hyphopodia).

Hyphal aggregations and tissues: During certain stages of life cycle, fungal mycelia become organized loosely or compactly that form some structures called *plectenchyma* (i.e. woven tissue). Its two general types are known as *prosenchyma* (i.e. approaching a tissue) and *pseudoparenchyma* (a type of plant tissue). These two types compose various other somatic and reproductive structures like *stroma* (mattress), *sclerotium* (hard structure) and *rhizomorph* (root shaped).

Reproduction in fungi: Fungi reproduce by three processes viz., (A) Vegetative, (B) Asexual and (C) Sexual reproduction.

Vegetative reproduction

a. Fragmentation
b. Fission
c. Budding
d. Sclerotium
e. Rhizomorph
f. Chlamydospores
g. Oidia (small egg)

Exogenous: The spores (reproductive units) borne at the tip or outside the vegetative structure called conidia (sing. conidium i.e. dust). Two types of conidia are thallospores and conidiospores. The letter has got three types viz., Blastospores, Aleuriospores, and Phialospores. The bearing structure is called conidiophore (phore=bearer). Generally,

conidia are developed on a simple (without branch) tubular conidiophore. Some other types of conidia bearing structures are phialids (small bottle type), synnema, coremia, acervulus (heap), sporodochia, pycnidia and sori (sing. sorus) or pustule.

Endogenous: The spores produces in sporangia (sing. sporangium; vessel or container) and hence called sporangiospores. Sporangiospores are of two types viz. (i) plasmospores or zoospores or swarm spores which are motile due to having flagella and (ii) aplanospores which are non-motile due to lacking flagella

Sexual reproduction

The sexual reproduction takes place by fusion of two compatible haploid nuclei, usually the gametes. There are two distinct fungal species

Monoecious or hermaphroditic: they are bisexual having both sex organs on one thallus (homothallic).

Dioecious: they are unisexual having either male or female sex organs on one thallus (heterothallic)

Four distinct phases of sexual reproduction are: somatogamy, plasmogamy, karyogamy and meiosis. These phases occur by any one of the following five general methods of sexual reproduction,

i. Gametic copulation – (a) Isogamy and (b) Anisogamy
ii. Gametangial contact
iii. Gametangial copulation
iv. Spermatization
v. Somatogamy (Anastomosis)

Classification of Fungi, Taxonomy and Nomenclature

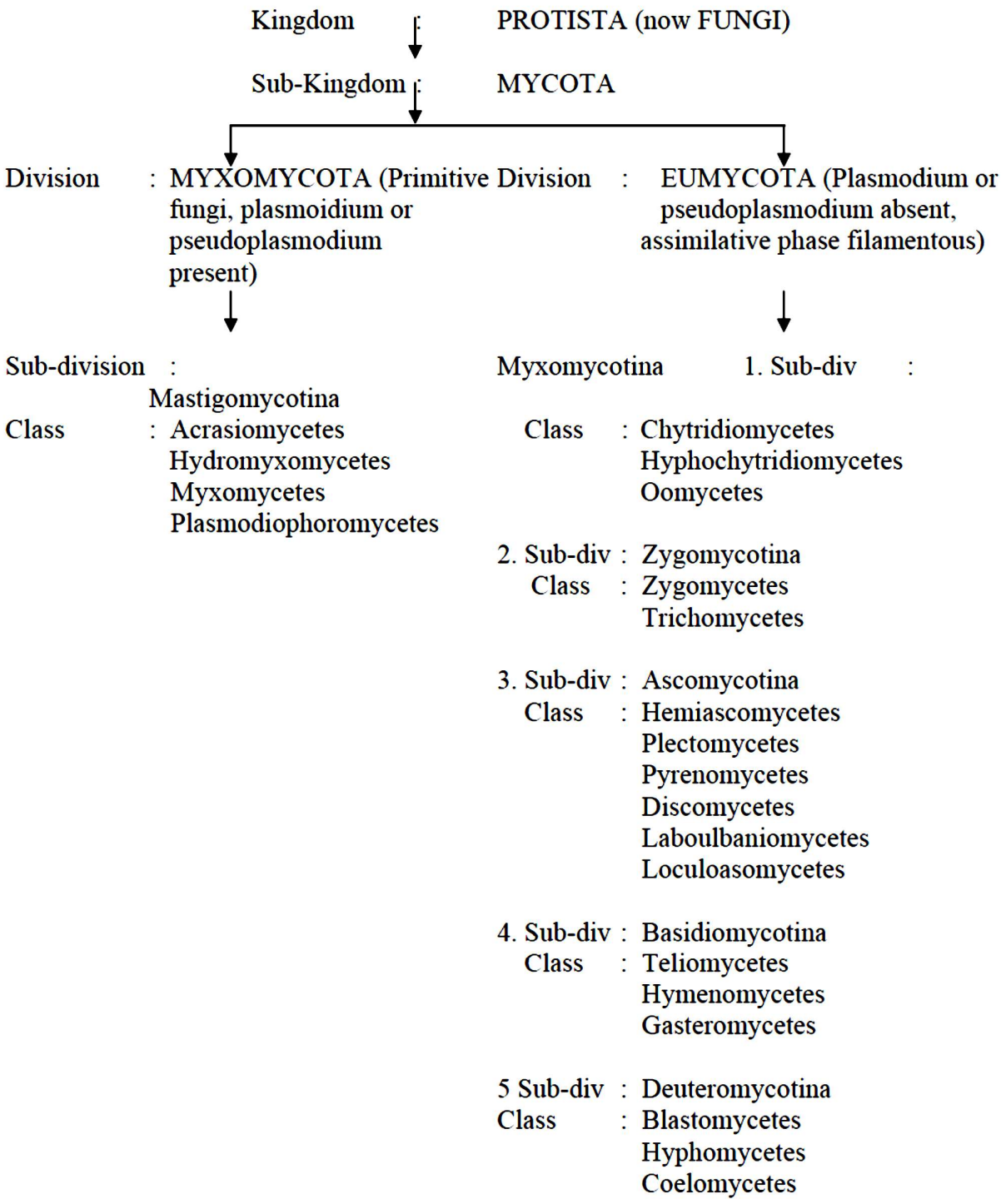
A. Taxonomy and units of Classification:

Super kingdom - Eukaryonta
Kingdom - Protista (now Fungi)
Sub-kingdom - Mycota
Division - mycota (suffix)
Sub-division - mycotina (suffix)
Class - mycetes (suffix)
Sub-class - mycetidae (suffix)
Order - ales (suffix)
Family - aceae (suffix)
Genus -
Species -

Suffix are used or added to the scientific names of a taxon. A taxon (pl. taxa) is a category in the classification system. Whittaker (1969) provided five kingdom system viz., Monera, Protista, Plantae, Animalia and Fungi, and thus Fungi is separated from Protista on the basis of nutrition pattern.

B. Various classifications

Classification of fungi was given by various authors viz. Gwynne-Vaughan and Barnes (1927), Martin (1931, 1941), E.A. Bessey (1950). C.J. Alexopoulos (1962) etc. The classification forwarded by Ainsworth (1966 and 1972) is most widely accepted that has been given below:



Nomenclature

Naming of fungi and their classification fall under the rule of *International Code for Botanical Nomenclature*. The scientific name of an organism follows the pattern of binomial (bi = two+ nomen = name) nomenclature system, which is composed of two words. The first word designates the genus and the genus name is always capitalized. The second word designates the species and its name is not capitalized. Binomials when written are underlined and when printed italicized. Modification or updating, if any, in the nomenclature is done by a Committee for Fungal Nomenclature at each International Botanical Congress held at every four years interval

- Exposures at 550 ppm for 15-30 min causes colic diarrhea and bronchial pneumonia.
- Chief source of H_2S are decaying vegetation, animal matter, sulphur springs and volcanic eruption.

NITROGEN OXIDES→ N_2O, NO, NO_2

About 95% of NO_x emitted as NO and 5% as NO_2.

❋ N_2O has so far not been implicated in air pollution problems.

❋ NO Chief source is industries manufacturing HNO_3, automobile exhausts. It forms several secondary pollutants like PAN, O_3 carbonyl compounds etc.

NO_2

* Deep reddish brown gas.
* Only coloured pollutant gas.
* Chief constituents of photochemical smog.
* Causes irritation of alveoli → emphysema.
* Lung inflammation → edema → death.
* Sensitive plant show visible leaf injury when exposed to 4 to 8 ppm for 1 – 4 hours.

OZONE (O_3)

Ozone layer in the stratosphere protects us from the harmful UV radiation from Sun.
On the other hand ozone is also formed in the atmosphere through chemical reactions involving certain pollutants on absorption of UV radiations.

Ozone: the destroyer →

Ozone and other oxidants such as PAN are formed by light dependent reactions between NO_2 & hydrocarbons. These pollutants cause **photochemical smog.**

❋Increase in O_3 → decrease crop yield

❋In plants O_3 enters stomata → produces damage to leaves → decrease in yield

❋O_3 hardens rubber reaction with many fibres like cotton, nylon & polyester.

Ozone :- the protector

1. Protect us from the harmful UV-radiations of earth.
2. Ozone by absorbing UV radiations heats up the stratosphere and causing temperature increase.
3. Some pollutants enter the stratosphere and remain there for years until they react with ozone and deplete it.
4. Major pollutants are – **chlorofluorocarbons, nitrogen oxides and hydrocarbons.**

* Depletion of O_3 would lead to serious temperature changes on earth and consequent damage to life support systems.
* Temperature changes and rainfall failure on earth.
* Cause cancer, especially relating to skin like melanoma.
* other disorders are cataracts, destruction of aquatic life, vegetation and loss of immunity.

Global efforts:

* Ist global conference on the depletion of ozone layer was held in Vienna (Austria) in 1985. hole on South Pole was discovered.

*** Montreal Protocol (1987) → 50% cut in CFCs**

➡ **The Montreal Protocol on Substances That Deplete the Ozone Layer is an international treaty designed to protect the ozone layer by phasing out the production of a number of substances believed to be responsible for ozone depletion. The treaty was opened for signature on September 16, 1987 and entered into force on January 1, 1989**

➡ **Kyoto protocol**: The treaty is intended to achieve "stabilization of greenhouse gas concentrations in the atmosphere at a level that would prevent dangerous anthropogenic interference with the climate system. The Kyoto Protocol establishes legally binding commitments for the reduction of **four greenhouse gases (carbon dioxide, methane, nitrous oxide, sulphur hexafluoride)**, and two groups of gases **(hydrofluorocarbons and perfluorocarbons)**

*** In June 1989, two Japanese companies claimed to have developed an alternative of CFCs. The device called as ice cleaning, is a semiconductor-washing device, which uses fine particles of ice and frozen alcohol at temperature below -50°C.**

E. Fluorocarbons:-

Fluorides in atmosphere come from industrial processes of phosphate fertilizers, ceramics, aluminium, fluorinated hydrocarbons. In plant it cause **tip burn**.

F. Hydrocarbons:-

Chief pollutants are→ Benzene, benzopyrene & methane

Chief source→ motor vehicles.

* Carcinogenic effort on lungs.
* Combine with NOx to form pollutants like PAN, NOx etc.
* Benzene cause lung cancer
* At increase levels methane in absence of O_2 may be narcotic on man.

G. METALS: -

Common are → Hg, Pb, Zn, Cd.

➡**Mercury** → Volatile metal

* Inhalation of 1 mg/m^3 for 1 month may lead to death.
* Nervous system, liner, eyes are damaged.
* Infant may be deformed.
- Headache, fatigue, anxiety, lethargy, loss of appetite.

Minimata disease is a neurological syndrome caused by severe mercury poisoning. Symptoms include ataxia, numbness in the hands and feet, general muscle weakness, narrowing of the field of vision and damage to hearing and speech. In extreme cases, insanity, paralysis, coma and death follow within weeks of the onset of symptoms.

➡**Lead: -** lead level of air by WHO→ 2µg/m^3 inhalation causes→ reduced hemoglobin formation damage RBCs, infection of liner and kidney.

✳ **Lead accumulates in the bones and lead poisoning may be diagnosed from a blue line around the gums. Lead is especially harmful to the developing brains of fetuses and young children and to pregnant women. Lead interferes with the metabolism of calcium and Vitamin D. High blood lead levels in children can cause consequences which may be irreversible including learning disabilities, behavioral problems, and mental retardation. At very high levels, lead can cause convulsions, coma and death**

➡**Zinc:** - Zn in air mostly occurs as white ZnO fumes and is toxic to man.

➡**Cadmium**: - Industries engaged in extraction, refining electroplating and welding of cadmium containing materials Cd is poisonous at very low levels and accumulates in liver and kidney. Causes hypertension, emphysema and kidney damage.

Itai-itai disease ("ouch-ouch" disease) was the documented case of mass cadmium poisoning in Toyama Prefecture, Japan. The cadmium poisoning caused softening of the bones and kidney failure. The disease is named for the severe pains caused in the joints and spine. The term **itai-itai** disease was coined by locals

H. PHOTOCHEMICAL PRODUCTS

Olefins, aldehydes, ozone, PAN, PB_2N & PHOTOCHEMICAL SMOG.

Olefins- exhaust from ethylene. At very low concentration they affect plants seriously, wither sepals, dropping of petals, retard opening of flowers.

Aldehydes- acroloin irritate the skin, eyes and upper respiratory tract.

Benzypyrene is carcinogenic.

PAN – eye irritant, respiratory distress, death of forest trees by blocks hill reaction in plants.

Photochemical smog: - constitutes O_3, NOx, H_2O_2, PAN etc. formation occurred only during night or cloudy days. Affect plants, human health and material.

* enter as part of inhaled air → irritation in respiratory tract.
* Emphysema is caused
* reduce visibility, damage crop & livestock, cause corrosion of metals, stones, building materials, textile, paper, leather etc.

I. PARTICULAR MATTER-

Discrete mass of any material exists as liquid or solid in atmosphere and of microscopic or submicroscopic dimension.

primary **particulate matter** plasma membrane includes dust, smoke particles.

four sources of **particulate matter** plasma membrane-

1. Fuel combustion and industrial operations.
2. Industrial fugitive processes
3. Non industrial fugitive processes
4. Transportation sources.

* When inhaled cause respiratory disease, tuberculosis and cancer.

**** Cotton dust causes occupational disease Byssinosis***

Byssinosis, commonly called "Brown Lung", is an occupational lung disease caused by exposure to cotton dust in inadequately ventilated working environments. It commonly occurs in workers who are employed in yarn and fabrics manufacture industries. Brown Lung can ultimately result in narrowing of the trachea in the lungs, destruction of lung tissue and death from infection or respiratory failure.

J. TOXICANTS

Arsenic: - by product of metal refining process and cause cancer.

Asbestos:- mineral fibre used in asbestos cement pipes flooring products, cement sheets etc. These fibres are non-degradable, cause cancer in man.

Carbon tetrachloride and chloroform – used making for CFCs refrigerants and propellants etc. have carcinogenic effects.

Chromium:- used in stainless, tool and alloy steel have carcinogenic effect.

Nickel:- used in chemicals, petroleum & metal products. In organic Nickel is strongly carcinogenic in man.

Vinyl chloride:- prime compound of Poly vinyl chloride (PVC). Known carcinogen in man. Induce brain & lung cancer.

Prevention and Control:- Vehicular pollution.

1. check pollutant emission from vehicular exhaust 2. control evaporation from fuel tank
3. use of filters 4. control through law.

Industrial Pollution

To remove particulate matter & gaseous pollutants, the equipment used are- cyclone collectors and electrostatic precipitators (ESPs).

Acid Rain: In the early 1970s it was noticed that lakes without any known source of acid in Canada, the U.S. and Scandinavia were becoming increasingly acidic and that the fish populations of these lakes were being depleted. Acid from the sky was the only explanation, and indeed, monitoring demonstrated that the acidity of rainfall was well above the natural acidity of rain. **This became known as acid rain.** In fact, the term acid deposition is more accurate, as acid-forming materials may be deposited from the air in the form of snow, sleet, fog as well as in the form of rain. Acid rain should have been no surprise. For over a century we have been burning large quantities of oil, coal & smelting ore to a lesser extent oil contain sulfur. In the presence of oxygen and high combustion temperatures, sulfur compounds are oxidized to become sulfur oxides (SO_x). Sulfur dioxide is itself a poison, but it can also react with ozone, hydrogen peroxide and water vapour in the atmosphere to form sulfuric acid (H_2SO_4). Combustion at high temperatures in power plants and smelters also create oxides of nitrogen, mostly as atmospheric nitrogen combines with oxygen. Although nitric oxide (NO) is not very harmful as it does not readily dissolve, nitric oxide can combine with oxygen to form nitrogen dioxide:

$$2NO + O_2 \rightarrow 2NO_2$$

Nitrogen dioxide is similar to sulfur dioxide, through various reactions with substances in the atmosphere, nitrogen dioxide is converted into nitric acid (HNO_3). Acid deposition has recently begun to cause obvious damage to the animals and plants in susceptible waters and soils where rain falls most heavily.

ACID RAIN

Most natural systems are slightly acidic. **Even rainfall in the absence of any industrial contamination is slightly acidic with a pH of 5.6, because of the formation of a weak solution of carbonic acid in the atmosphere, when carbon dioxide reacts with water**. **If it rains with a pH of 5.5 or less, it is called acid rain.** The pH of the acid rain vary in different areas, depending upon the industrial activity in an area and pH as low as 1.5 has been recorded in West Virginia,USA.

The English scientist, Robert Angus Smith coined the term "**Acid rain**" in the 1850's, while he was investigating the air chemistry of Britain's industrial heartland. The pH scale runs from 1 to 14 with pH 7 being the neutral. pH 1 represents highly acidic and pH 14 represents highly alkaline. Since the number of hydrogen ions varies greatly from one solution to another, the scale of acidity i.e. the pH of a substance is calculated on a logarithmic scale. A change of 1 pH unit is equivalent to ten-fold change in the number of hydrogen ions and is expressed as

the negative logarithm of the number of free hydrogen ions. The pH of milk, tomato juice and vinegar is 6.5, 4.2 and 2.8, respectively.
Since the pollutants (compounds of SO_2 and NO_x) not only mix with rainwater but also with fog, snow, dew and hail, it is better to call it acid precipitation. Some workers call it **acid deposition**, which is further distinguished into dry and wet deposition. In **wet deposition,** falling pollutants mix with water. If pollutants fall to the surface in the form of dry gases or adsorbed on **aerosols** like soot or flyash, it is called **dry deposition**. Dry deposition usually occurs near the source of pollution and takes 2-3 days to settle. Acid deposition results from atmospheric pollution. Man has been polluting the atmosphere since the Stone Age when he used to live in caves. Mans activities especially in the past century have increased the pollutants in the atmosphere. Once in the atmosphere, the winds carry the primary pollutants like SO_2 and NO_x away from the source depending upon the velocity of wind, and these pollutants then form secondary pollutants like sulphuric acid and nitric acid and their salts in the atmosphere.

SOURCE OF SULPHUR DIOXIDE AND NITROGEN OXIDES

These pollutants enter the atmosphere in two different ways:

1. Natural source: - It includes the volcanoes, lightening, burning of biomass and microbial activity and they are cosmopolitan in distribution. SO_2 and NO_x have been natural components of the atmosphere since the earliest times but have been in rare concentrations and levels of their natural emissions have remained more or less constant in the last twenty years or so.
2. Anthropogenic source: - Sulphur dioxide and nitrogen oxides enter the atmosphere in large quantities from the industrial regions of the world Nitrogen oxides are released by the combustion of gasoline and inspite of the use of catalytic converters, some escape to the atmosphere and vehicular emissions are the major source of these gases in the atmosphere.

Emission of sulphur dioxide is associated with burning of coal in electricity generation, smelting of iron ore and as a fuel for domestic heating. It is a major contributor of these pollutants to the atmosphere. The acid rain does not recognize any geographic boundaries. The pollutants remain in the atmosphere for about two weeks before settling to the earth's surface. The polluting chemicals from the source i.e. power plants and factories are carried by wind movements to the neighbouring countries without any obstructions. Thus Acid deposition in Norway, Switzerland, Austria, Sweden, Netherlands and Finland could be due to acidic emissions from the industries in UK and Germany. In the late 1960s, Swedish scientist Svante Oden found that pollutants from Europe and Great Britain were responsible for the acidification of lakes in Scandinavia. It is believed that Asia suffers from worst acid deposition where China alone gets 59% of its energy from the burning of coal ***EFFECTS OF ACID RAIN:*** Acid rain is harmful and affects every segment of the environment . Some of the effects of acid rains are listed below:

1.**Human health**:

a. In dry gaseous or aerosol forms, acid rain causes respiratory problems. Sometimes, it can also lead to death. The pollutants are generally localized in origin. The London Smog of 1952 resulted in 4000 deaths because the people inhaled sulphuric acid, which damaged the lungs and aggravated the breathing problems.
b. Man is also affected indirectly through biological concentration of certain metals like lead, copper, cadmium, zinc and mercury along the food chain. These metals are liberated free from their compounds in soil by reaction with sulphuric (H_2SO_4) and nitric acid (HNO_3) deposited in the soil by acid rain. They reach the humans through plants via food chain concentration or drinking water supplies. Storage tanks and water pipes are also corroded by acidified water and add metals to the drinking water. Lead from lead pipes and soldering of the joints is very harmful to human health.
c. Acid rain may cause itching or burning sensation if it falls on the body.

2.**Terrestrial environment**

a. Acid rain affects the plants directly. At high altitudes, the trees remain surrounded by clouds for long periods and show necrosis and degradation of chlorophyll. In the plains, the acid rain falls on the trees and affects its different parts eg. leaves, flowers and fruits and damage the plants causing necrosis, chlorophyll degradation and leaching of the nutrients. Acid water interferes with soil biology and soil chemistry disturbing nutrient cycles and causing physiological damage to plant root system. Acidity may affect the activity of nitrifying bacteria to fix atmospheric nitrogen.
b. It weakens the plants and makes them more susceptible to cold, diseases and insect attacks.
c. It promotes growth of mosses, which have high water holding capacity, killing the roots of the plants and fungi that help the roots in the absorption of nutrients.
d. Acid deposition helps in the release of certain ions like Al^{3+} from insoluble form and these ions inhibit the uptake of water and nutrients from the soil by the plants. Increased acidity in the soil precipitates phosphorous, as insoluble compounds and they cannot be absorbed by the plants as nutrients. The rate of decomposition is also slowed down due to the death of the decomposers.
e. The toxic metals like Al, Cd, Zn, Hg, Cu and Fe released in ionic form due to acidification may mix with ground water, lakes and streams or get absorbed by plants and damage them. Low concentrations of iron are useful for the plants but at higher concentration iron is toxic to them.

3.**Aquatic environment**

a. The lakes get acidified either due to ageing or acid rain and it has a declining effect on the flora and fauna. The poisoning of

the food chain starts with the photosynthetic algae and once the food base is affected, the whole system is likely to collapse. Phytoplankton disappears below pH 5.8. However, the lake will be invaded by *Sphagnum* mosses, which are acid tolerant. The acid lakes are unusually clear and bluish in colour in the absence of phytoplankton and reduced organic activity. They allow more light penetration.

b. Fishes show different degrees of tolerance to acidity eg. Brook trout cannot tolerate pH below 6 and the salmons are much less tolerant. So, the composition of fish population in a lake changes with acidity. The low pH of the lake helps in the release of unavailable metals like aluminum and mercury from the nearby soil into the lake, which is lethal to the fishes. In the presence of these metals, the fishes secrete excessive mucous which asphyxiates them by clogging the gills. Water bodies that show decline in fish populations are called dead or dying. It is believed that in Norway and Sweden about 1600 lakes are without fish due to increased acidity and 1400 lakes in Canada have negligible number of fishes.

c. Water boatman and Whirligig beetles are known to survive at pH 3.5 while some protozoan species survive even at pH 2.

d. **Built up structures** Weathering of rocks occurs due to physical factors like temperature and humidity or chemical factors like sulphur and NO_x compounds in the atmosphere. The acid rain corrodes the rocks gradually. However, the effect of acid rain is more on limestone and marbles, which are commonly used in the construction of buildings. Limestone has calcium and magnesium as main constituents and reacts with H_2SO_4 to form sulphates. Slowly, the building is damaged as the sulphate is washed out of the stone. The crystals of sulphates formed on or below the surface cause cracking, flaking and crumbling of the structure. Though, granite and bricks are resistant to acidity, it damages the lime rich mortar, weakening the brick built structures, steel and other materials in the building. The damages in urban areas are generally due to dry deposition and the acidic particles damaging the buildings originate from nearby smelters and power stations. The oil refinery at Mathura is a cause of concern for the Taj Mahal at Agra. Thus, acid rain apart from causing physical and economical damages, threatens the World's Cultural Heritages like the Taj Mahal in India, 37 feet bronze Buddha in Kamakura, Japan and the Cathedrals of Cologne, Cantabury in Europe. The craftsmanship on medieval stones of these buildings may be damaged beyond repair. Faceless statues and falling cornices of the worlds famous buildings are very common in acid rain affected regions of the world.

SOLUTIONS

The solution to the problem of acid deposition lies in controlling the concentration of its constituents in the atmosphere. This can be achieved in the following ways:

1. Reduction in the emission level of SO_2 and NO_x by improving energy efficiency.
2. Reduction in the use of fossil fuels and utilizing alternative source of energy like solar and wind energy.
3. By promoting use of low sulphur fuel like oil and natural gas.
4. SO_2 level in the coal can be reduced by fuel desulphurization and by crushing and washing the coal which removes 8-15% of the sulphur compounds.
5. Tax emissions of the pollutants like SO_2.
6. Remove NO_x from automobile exhaust.
7. Old and acidified lakes can be neutralized by adding limestone and thus made productive again.

THE STRATOSPHERIC OZONE

The earth's atmosphere is divided into several layers. Each layer is characterized by a sharp change in temperature due to differences in the incoming solar energy. The innermost layer is **troposphere**, which extends upto 17 km and has 80% of the earth's air. The tropospheric air has 78.08% nitrogen (N_2), 20.94% oxygen (O_2), 0.035% carbon dioxide (CO_2) and 0.934% argon apart from water vapour and trace amounts of other gases.

The second layer of the atmosphere is **stratosphere** and it extends from 17 to 48 km above the earth's surface. It is quite different from troposphere in the composition of its gases, as it has very small amount of water vapour and a high concentration of ozone. The stratospheric ozone layer helps in absorbing solar radiations entering the troposphere to different extent. The solar radiation has different components and includes the visible light, the UV light and the infrared radiations apart from many other radiations. The UV radiations are of shorter wavelengths and lie near the violet light of the visible spectrum, which are the shortest wavelengths reaching the eyes. The three main components of the UV radiations are UV-A with wavelength 320-400 nanometers, UV-B radiations with 280-320 nanometers wavelength and UV-C with wavelength 100-280 nanometers is lethal to organisms. Since the energy is inversely related to the wavelength, UV-B radiations are more energetic and dangerous.

As mentioned above, when these radiations enter the earth's atmosphere, the stratospheric ozone absorbs 95-99% of the UV radiations. If the full amount of these radiations reaches the earth's surface, it will be lethal to all forms of life. Even small amounts of UV radiations reaching the earth's surface can cause sunburn, skin and eye cancer and cataracts and may damage the immune system. It can also damage plants, increase the rate of any oxidative process like rusting of metal and is a major contributor to the photochemical smog. The key to the paradox of "love – hate" relationship with ozone lies in where the ozone is formed in the atmosphere. The ozone that we need is stratospheric ozone while tropospheric ozone is a problem gas. The stratospheric ozone protects us from the harmful effects of UV radiations and is rightly called the global sunscreen, the UV filter or the ozone shield. The protection of this stratospheric ozone is linked to the survival of mankind.