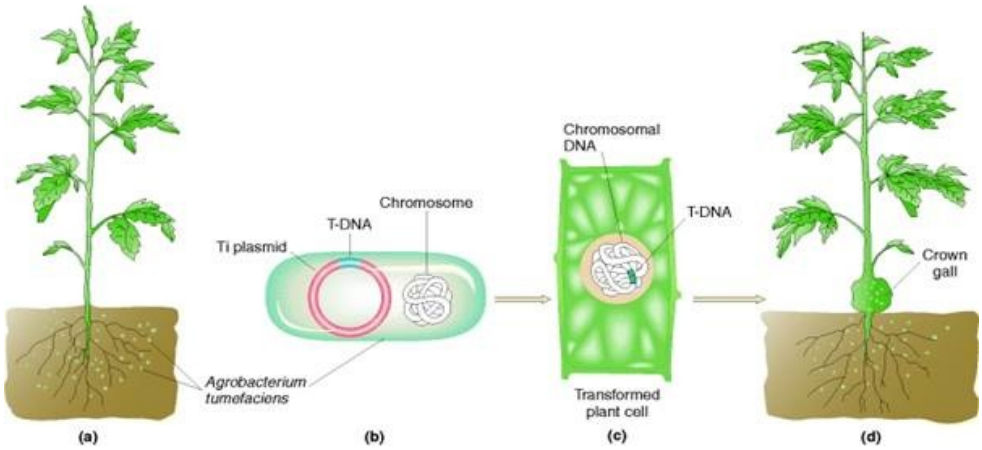
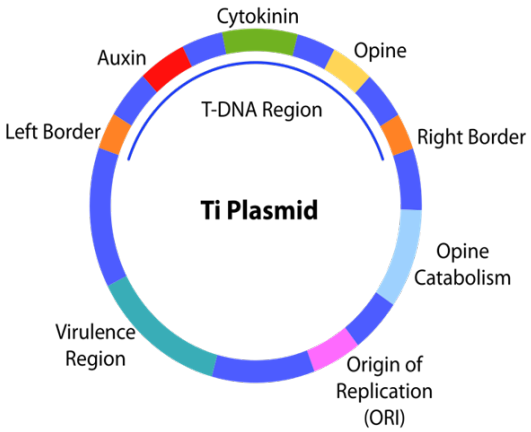
BIOLOGY OF AGROBACTERIUM MEDIATED GENE TRANSFER

- *Agrobacterium tumefaciens* is a rod shaped, gram-negative bacteria found in soil. This bacterium is known as natural genetic engineer because of its ability to integrate its plasmid gene into plant genome.
- It is the natural causative agent for crown gall disease.



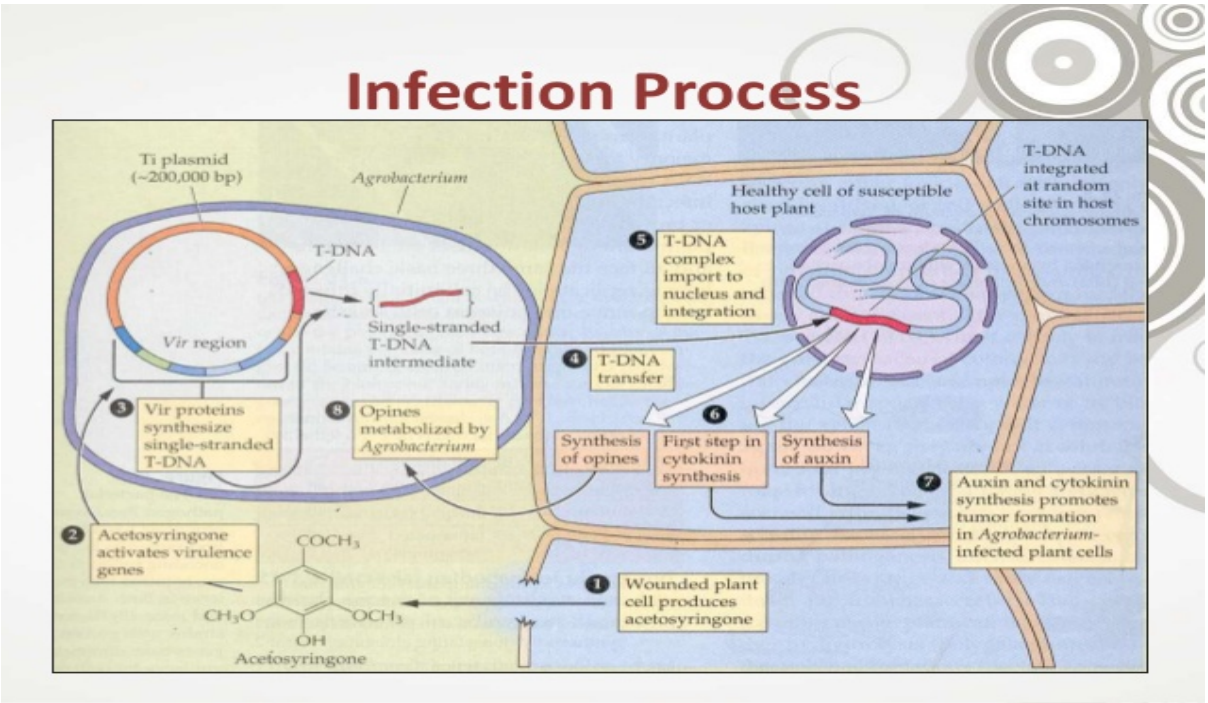
- *Agrobacterium tumefaciens* enters the plant through cuts or wounds present in its root or stem.
- The bacterium is attracted by phenolic compounds such as acetostringone released by the wounded plants. The bacteria sense these molecules (acetostringone) and accumulate near the wound. It comes in contact with the plant cells which have their genome within the nucleus.
- The bacteria have chromosomal DNA and an extra chromosomal DNA in the form of **Ti Plasmid.** The Ti Plasmid has many regions as indicated in the figure.



- It has a special segment called **T-DNA.**

- T-DNA region consist of many genes. Auxin and Cytokinin (plant growth hormones) producing genes and genes responsible for the formation of opine (nutrient source for *Agrobacterium*). A right border sequence which is 25 base pairs long and a left border sequence with variable length.

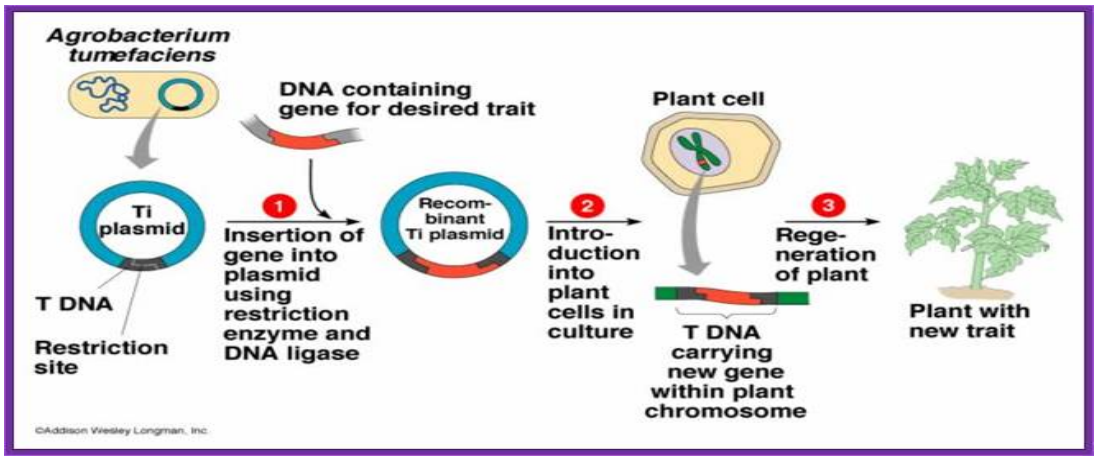
➢ When plant cell and bacterial cell come in contact at the wound region, acetosyringone released by wounded region activates virulence genes. They excise single stranded DNA and transfer it to the plant cell through a transport channel created between the two cells. The T-DNA complex import to nucleus and gets integrated into the plant cell genome.



➢ Plant cell has its own genome which has genes for the synthesis of auxin and cytokinin but when T-DNA gets integrated (which also has genes for production of auxin and cytokinin) both the genomes will transcribe resulting in over production of growth hormones and this over production leads to the synthesis of crown gall or tumor.

➢ The opines produced in the plant cells are metabolized by bacterium in contact.

➢ Due to presence of this feature scientists used this organism (technique) to transfer gene of interest into the plant genome through T-DNA region by modifying it (introducing a restriction site).



Nidhi Jarngal, Department of Botany, G.D.C Hiranagar.

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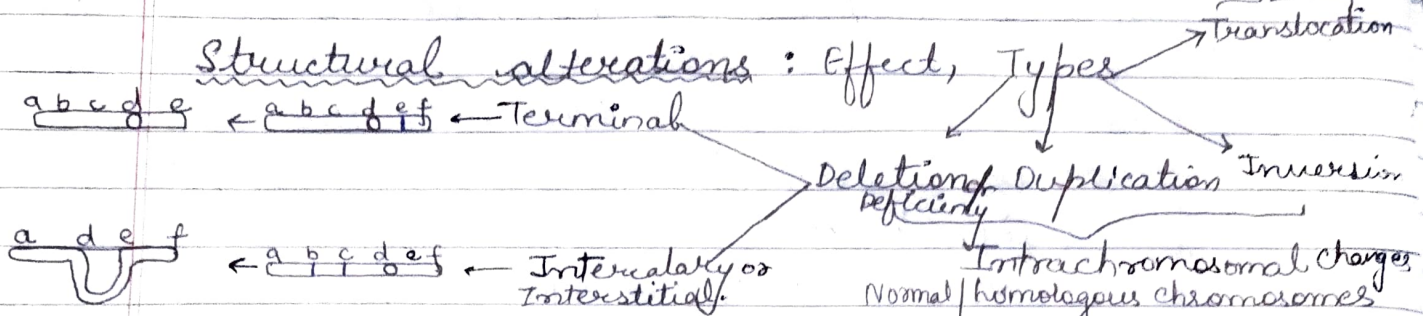
UNIT-IV

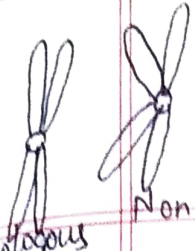
ALTERATIONS OF THE GENOME

Structural alterations : Effect, Types



The variation in the structure of the chromosome is called as chromosomal alteration. Mostly the higher organisms such as plants and animals, they are diploid in nature. The somatic cell of diploid organism contains two set of homologous chromosome. The gametes of these organisms contains single set of chromosome (haploid - N). The chromosomal alteration are of two types :- 1) Structural alteration (on the basis of structure)
2) Numberical alteration (on the basis of number)

Variation in chromosome structure :
Chromosomal mutation or chromosomal aberration :- The variation in chromosome structure occure due to breaking of chromosome so that the structural organisation as well as sequences of the chromosome/genes get disrupt. Sometime, the broken fragments reunite joined in some other ways or fused with some other segment. These structural changes are called as



Homologous chromosomes

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Non-Homologous Chromosomes.

chromosomal aberration If these changes are heritable in nature then they are called as chromosomal mutation. The chromosomal mutation may occur naturally or artificially The agents which causes to break the chromosome are radiations, (x-rays, γ-rays) nutritional deficiencies or through chemicals. The structural changes may be intra-chromosomal or inter-chromosomal. The structural alterations are of four types:

1) Deletion (loss or delete of chromosomal fragment).
2) Duplication
3) Inversion
4) Translocation

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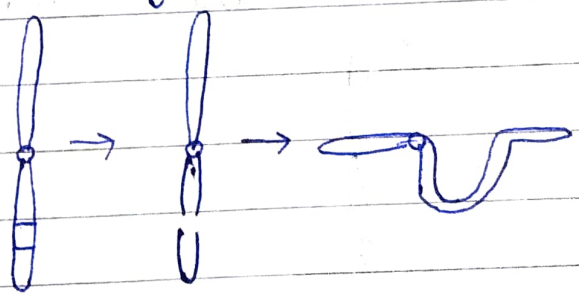
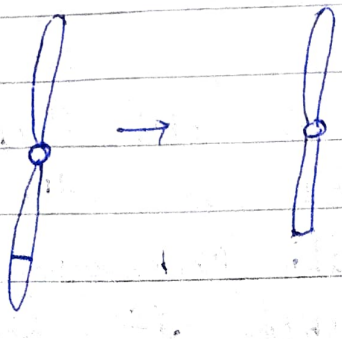
1) Deletion:- loss or delete of chromosomal fragment

Types :- 1) Terminal
2) Intercalary.

Intercalary:-



Terminal -



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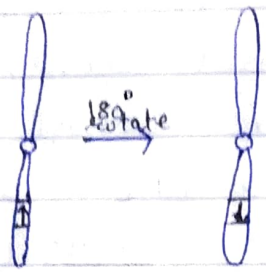
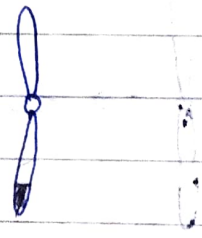
2) Duplication- Addition of fragment to the chromosome (homologous chromosome).

3) Inversion-



1) Deletion- Deletion is the most simple type of chromosomal alteration in which loss of chromosomal segment takes place. In this the segment break off from the main chromosome & gets lost. It is of two types :- 1) Terminal
2) Intercalary / Interstitial

1) Terminal → in which the loss of segment occurs from the end of the chromosome.



Terminal.

2) Intercalary - in this type the loss of segment occurs from the centre or middle portion of the chromosome. The deleted segment when it lack a centromere called acentric.

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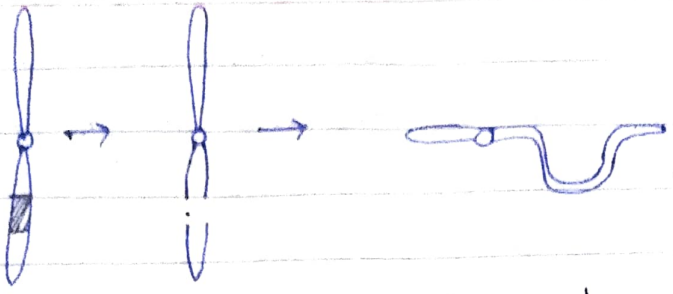


Intercalary.

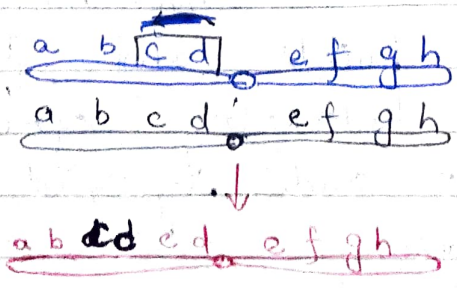
2) Duplication – Its simply mean that a part of chromosome is duplicated or addition of chromosome segment is called duplication In this type, a chromosomal segment is deleted from one chromosome and gets attached to other homologous chromosome. So that during the process of duplication the length of the chromosome increase.

There are many types of duplication :–

1) Tandem (Repeat)
2) Reverse
3) Displaced
4) Translocation.

★ Pallister Killen Syndrome
If extra addition to the 12 pairs of chromosome. Upper lip thin, lower lip thick. Short Nose, Hearing problems, Short life cycle (Not more than 40yrs.) Cataracts.

1) Tandem – In this type, deleted segment of one chromosome is directly added to adjacent chromosome to the same place so that gene order remain same but in repeated way.



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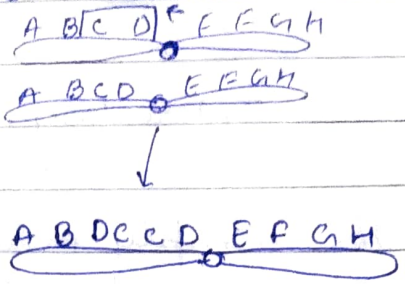
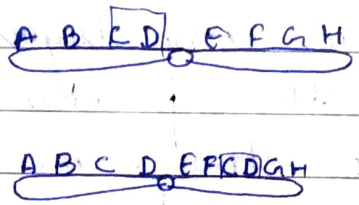
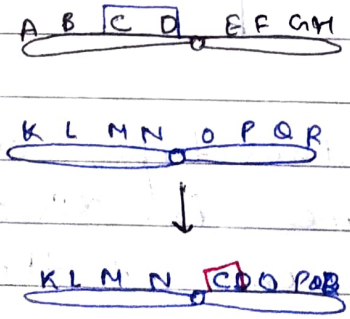
2) Reverse - In reverse tandem duplication is same but deleted segment is added in reverse order.



3) Displaced - In this type, the segment is added into different location.



4) Translocation - In this type, the segment is added to some other chromosome.



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3) Inversion → It is a type of chromosomal aberration in which chromosomal segment breaks at two points and forming four cut ends, the intercalary segment is turn through 180° and regained the same chromosome.

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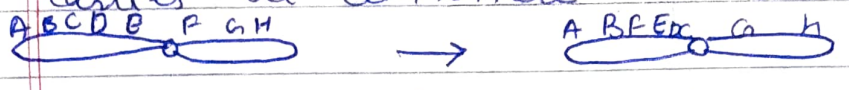
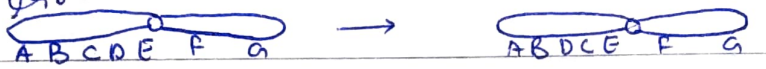
1 Inversion are of two types:
1) Pericentric
2) Paracentric

1) Pericentric - in which fragment of chromosome carries a centromere is called pericentric.

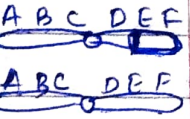


2) Paracentric - In case of paracentric type, in which fragment of chromosome does not carries a centromere.

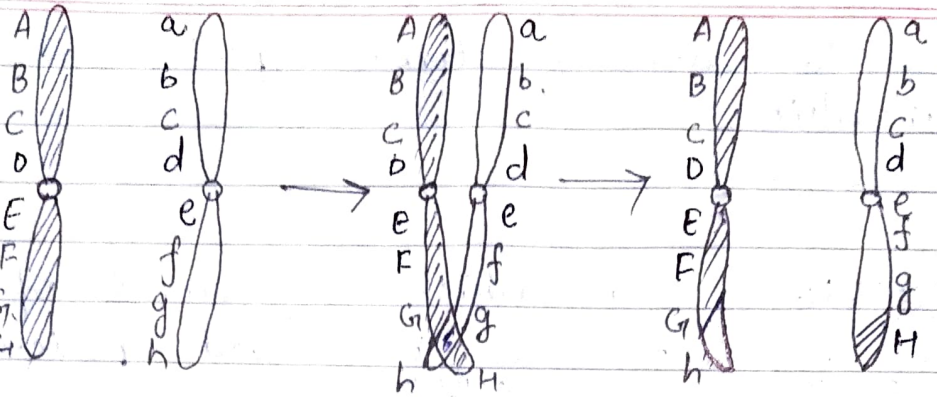


4) Translocation - In translocation, a segment of the chromosome is broken from one chromosome and joined to other (non-homologous) chromosome. So that both the chromosome gets modified. The donor chromosome becomes short due to deletion of fragment and the recipient becomes longer due to extra addition of genes. 3 types of translocation :-

1) Simple → In this type, a chromosome undergoes single break and releasing a terminal fragment. the broken fragment add to the same or some other chromosome, so that gene sequences changes.

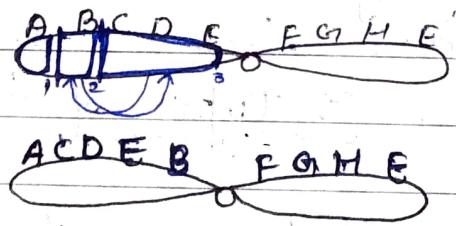


2) Reciprocal → In this type, the two non-homologous chromosome undergoes single break and the broken segment are mutually exchanged between them.



Recriprocal translocation is most common and is performed by two non-homologous chromosome.

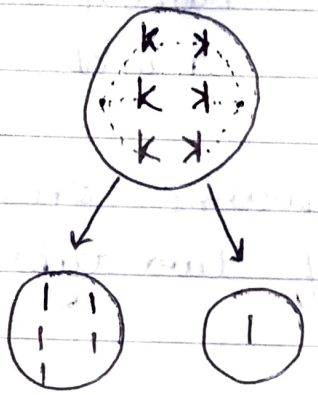
3) Shift → In this type, chromosome undergoes three break and producing six cut ends. The middle segment is shifted from its original position to a different position This process is also known as trans-position.



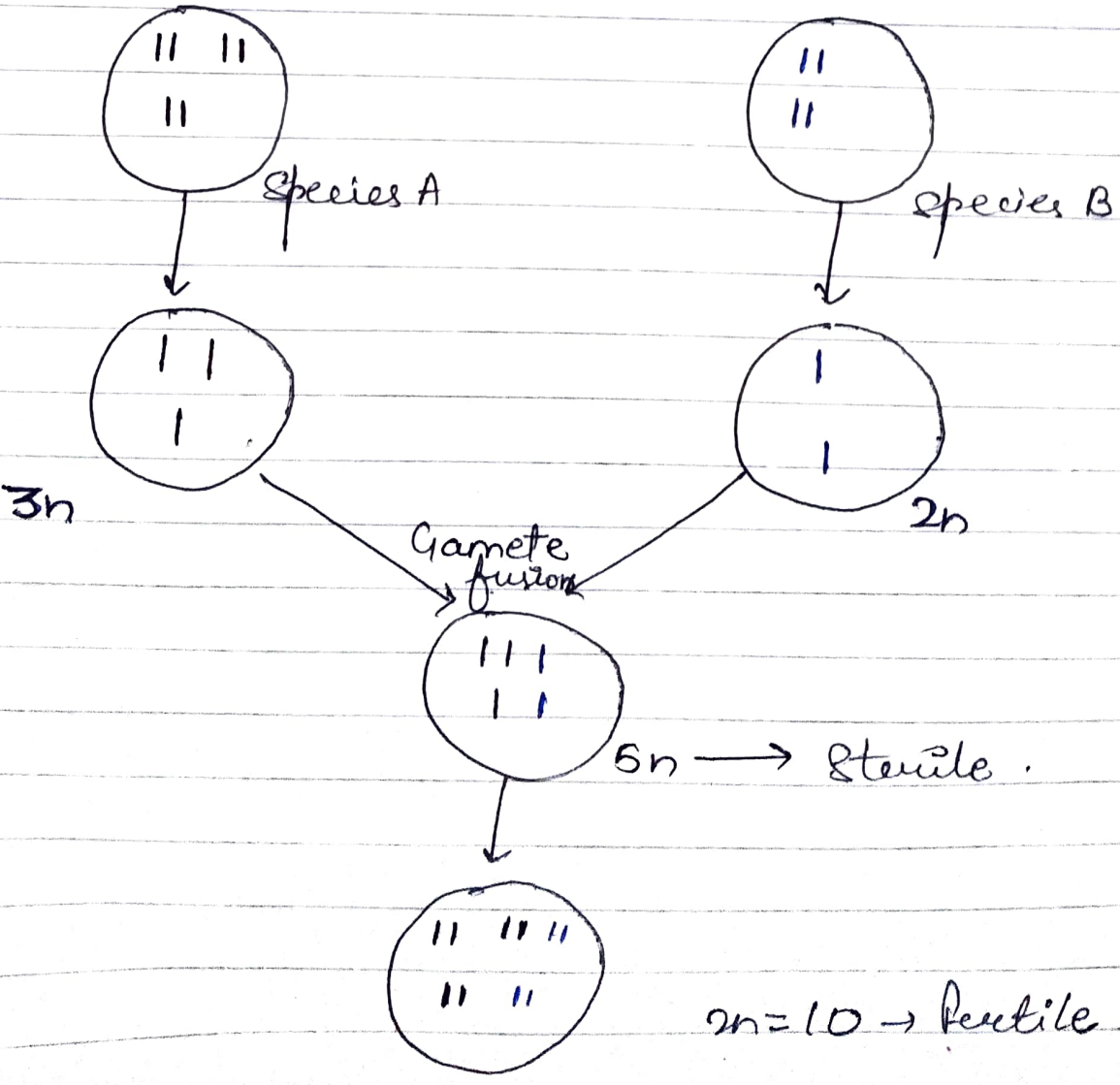
In case of pericentric, the size of chromosome changes because of centromere include within conversion but in case of paracentric the size of the chromosome remain same because of the centromere is exclusive.

Non-disjunction of chromosomes:

Mistake in chromosome saggregation.
OR
Failure of disjunction of chromosome.
Change in chromosome no by 1,
Change in chromosome no by 2.



Non-disjunction of chromosomes.



Ploidy (Complete set of paired chromosome / genome).

Aneuploidy (Presence of abnormal no. of chromosomes)

Hypoploidy
when the chromosome no. decrease from normal.

- One chromosome is missing → **Monosomy** (2n-1)
- Two chromosomes are missing, so that non-viable offspring are produced → **Nullisomy** (2n-2)

Hyperploidy
when the chromosome no. increases from normal.

- Addition of one chromosome → **Trisomy** (2n+1)
  - **Autosomal trisomy**
  - **Sex-related trisomy**
- **Tetrasomy** (2n+2)

Polyploidy (Multiple sets of paired chromosomes in the organisms / cell]

Eg. In Humans = 23
23 × 2 = 46 chr.

- **Allopolyploidy** (cell division takes place in same species)
- **Autopolyploidy** (Fusion of gametes of two diff. species)

Aneuploidy → Presence of abnormal no. of chromosome is called aneuploidy.

Eg In humans, 46 chromosomes in normal condition, either the presence of 47, 48, 49 or 44, 45, they are said to be abnormal or aneuploids.

From where does Aneuploidy arises?

It occures during cell division (non-disjunction of homologous chromosomes) spindle assembly disruption, spindle assembly check points disruption, deletion or translocation.

Monosomy - These are the different conditions which occur in human beings such as:

1) Turner's syndrome (XO) - In females - 45 chro. This syndrome is seen in female in which one X chromosome is missing.
2) Cri-du-Chat Syndrome - In this syndrome, deletion of chromosome's P arm of 5th chromosome.

Nullisomy - In this condition, two chromosome are missing, so that non-viable offsprings are produced after fertilisation.

Trisomy - Addition of one chromosome i.e. 2n+1. They have one extra chromosome.

Autosomy trisomy - 1) Down Syndrome (21)
2) Patau's Syndrome (13)
3) Edward's (18).

Sex-related trisomy - 1) Super Male (44+XXY)
2) Super female (44 XXX)
3) Klinefelter's Syndrome in Male.

Tetrasomy - 1) Extreme Super Male (XYYY)
2) Extreme Super Female (XXXX)

Colchicine reacts with spindle proteins of spindle fibres and inhibits its function which results improper saggregation

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of chromosome which leads to polyploidy.

Polyploidy

Autopolyploidy
Cell division takes place in same species

Allopolyploidy
There is fusion of gametes of two different species.

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Polyploidy.

Examples 1) wheat (Triticum aestivum) (Bread wheat)

2) Cotton (Gossypium)

Gossypium hirsutum commonly known as upland cotton.

Cotton Cross

Gossypium herbaceum × Gossypium raimondi

Old world cotton — American (upland cotton)

Diploid – 2n = 26, 13 (AA) — large size of chromosome

2n = 26 – Diploid, 13 (DD) — Small size of chromosome

F1 hybrid

(A D) 2n = 26 , 26 (Valents)

doubling of chromosome

13 large, 13 small

Artificially induced polyploidy – Colchicine

2n = 52

AA DD – Tetraploid

eg. hirsutum – Artificial synthesis of new world cotton.

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EVOLUTION OF WHEAT

It is an example of allapolyploidy. The genus Triticum consist of 3 different chromosome no's namely 2n=14, 2n=28, 2n=42, basic set x=7.

Common Name	section	Species	Genome Constitution
Wild einkorn	Monococcum	T. monococcum	AA - Diploid
Wild emmer cultivated emmer (Macroni wheat)	Dicoccoidea	T. turgidum T. dicoccum T. durum	AA BB - tetraploid
Common wheat or Bread wheat	Triticum	T. aestivum subspecies Compactum spelta	AABBDD - Hexaploid.

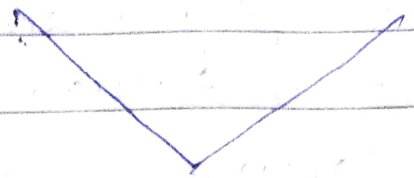
(Cultivated) Triticum monococcum (2n=14) AA — f1 hybrid — AB — Hybrid Doubling of Chromosome — Triticum durum AA BB - Tetraploid 2n = 4x = 28

(Wild goat grass) Aegilops speltoides (2n=14) BB

AABB × DD

Triticum durum — Aegilops squarrosa

2n=28 — 2n=14



ABD – Sterile

2n=21 – 2n=3x=21

Doubling of chromosome

AABBDD – Hexaploid

Triticum aestivum

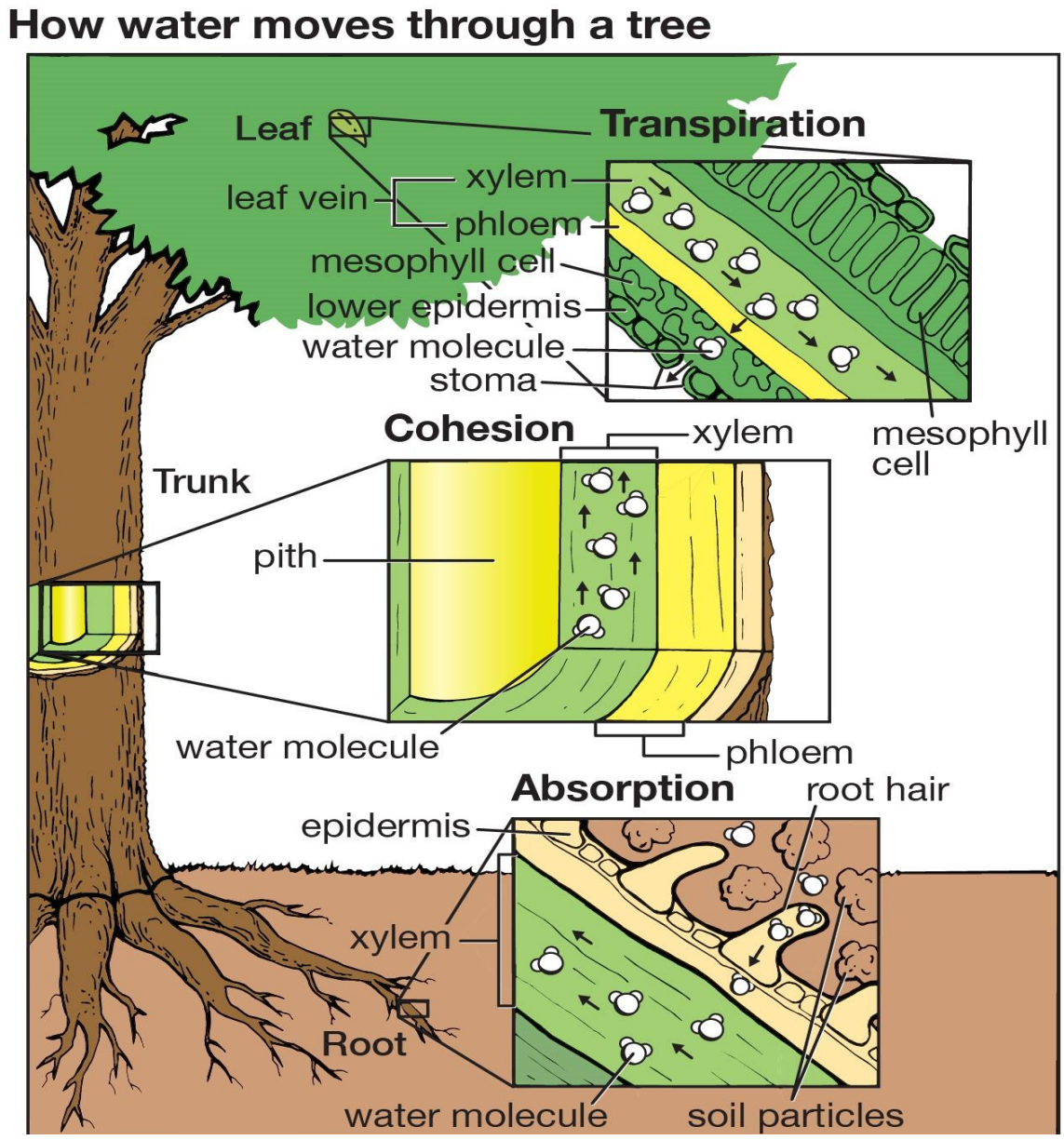
2n=42 ⇒ 2n=6x=42

Cultivated hexaploid wheat, commonly known as bread wheat.

ASCENT OF SAP

Anil Kumar Dogra
Assistant Professor (Botany)
GDC Hiranagar.

The water after being absorbed by the roots is distributed to all parts of the plants. In order to reach the topmost part of the plant, the water has to move upward through the stem.



The upward movement of water is called as Ascent of sap.

Ascent of sap can be studied under the following two headings.

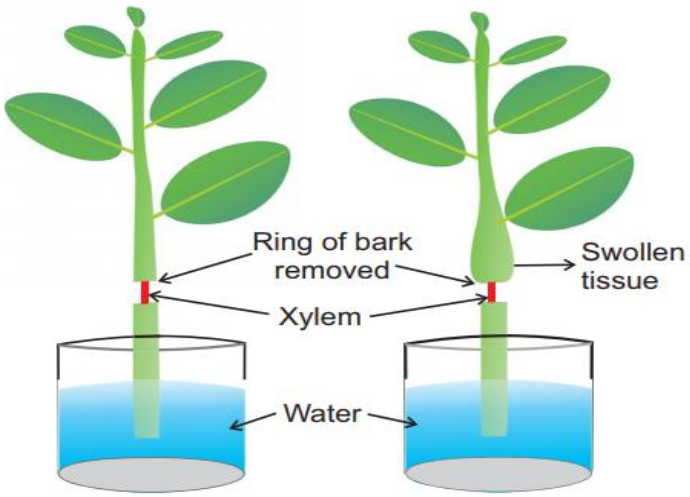
1. Path of ascent of sap

2. Mechanism of ascent of sap.

1. Path of ascent of sap

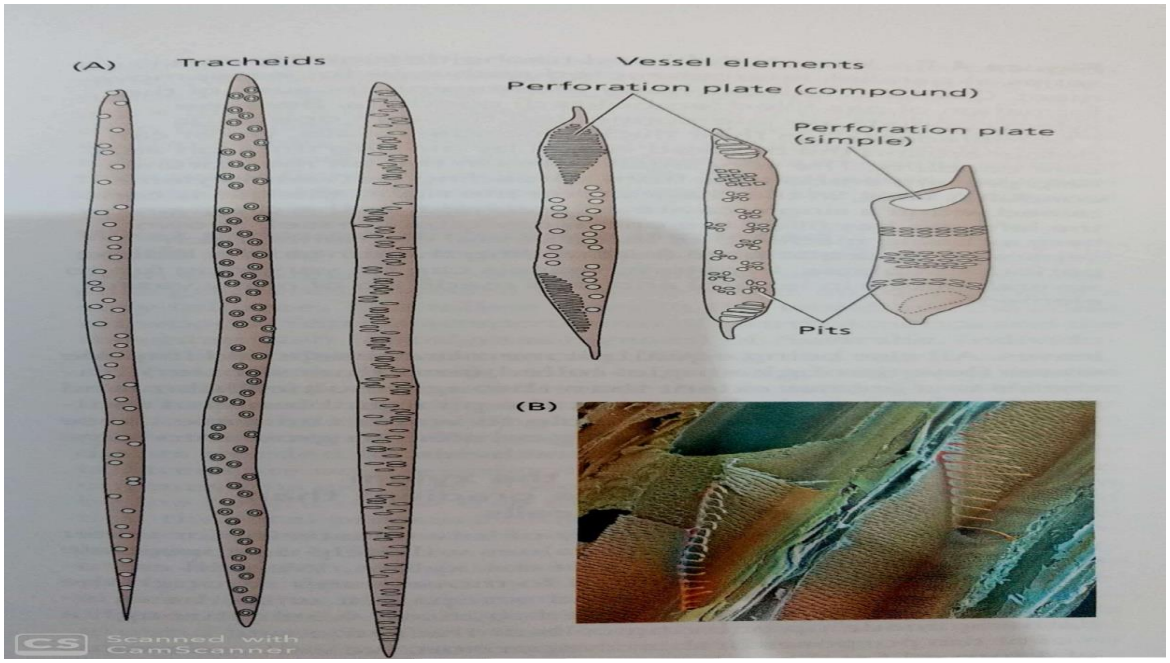
Ascent of sap takes place through xylem. It can be shown by the experiment. A leafy twig of **Balsam plant** is cut under water and placed in a beaker containing water with some Eosine (a dye) dissolved in it. After sometimes **coloured lines** will be seen moving upward in the stem. If sections of stem are cut at this time, only the xylem elements will appear to be filled with coloured water.

2. Ringing experiment



A leafy twig from a tree is cut under water and placed in a beaker filled with water. A ring of bark is removed from the stem. After sometime it is observed that the leaves above the ringing part of the stem remain fresh and green.

It is because water is being continuously supplied to the upper part of the twig through xylem.



Mechanism of ascent of sap

In small trees and herbaceous plants, the ascent of sap can be explained easily, but in tall trees like Eucalyptus and conifers reaching a height of 300-400 feet), where water has to rise up to the height of several hundred feet, the ascent of sap, it feet, becomes a problem.

To explain the mechanism of Ascent of sap, a number of theories have been put forward.

A. Vital Theory

B. Root Pressure Theory

C. Physical Force Theory

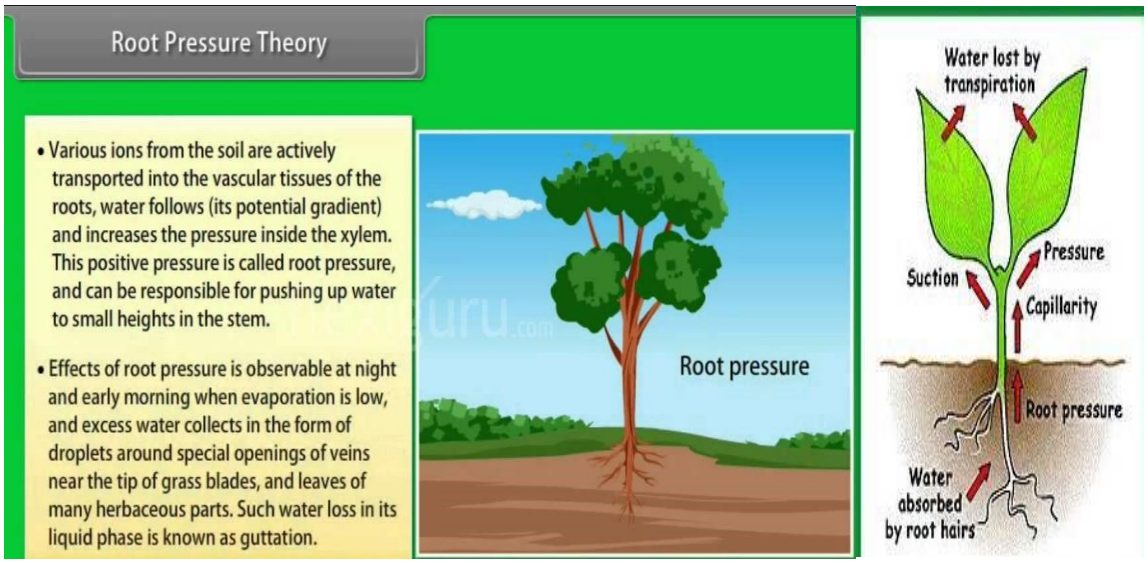
D. Transpiration Pull and Cohesion of Water Theory

A. Vital theories According to vital theories, the ascent of sap is under the control of vital activities in the stem.

1. According to Godlewski (1884) – Ascent of sap takes place due to the pumping activity xylem tissues which are living.

2. According to Bose (1923) – upward translocation of water takes place due to pulsatory activity of the living cells of the inner must cortical layer just outside the endodermis.

B. Root pressure theory Although, root pressure which is developed in the xylem of the roots can raise water to a certain height but does not seem to be an effective force in ascent of sap due to the following reasons. Magnitude of root pressure is very low.

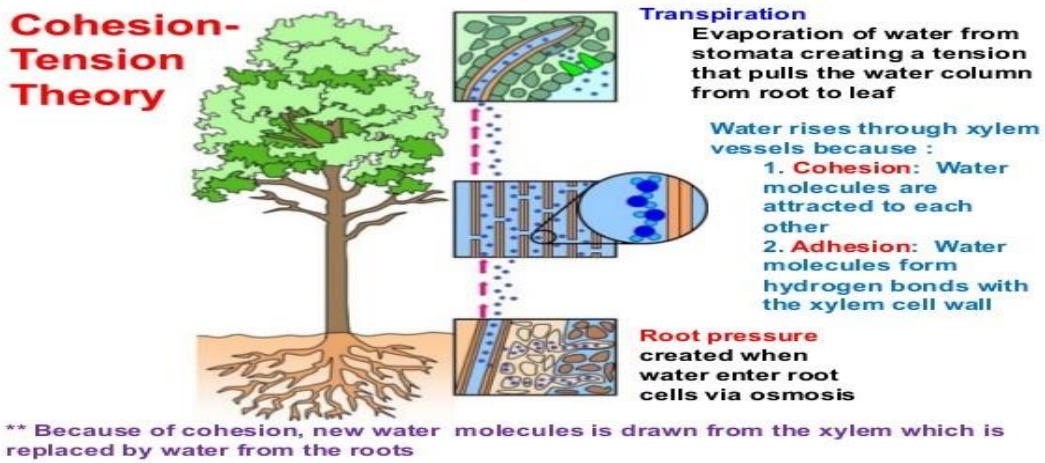


C. Physical force theories Various physical forces may be involved in ascent of sap.

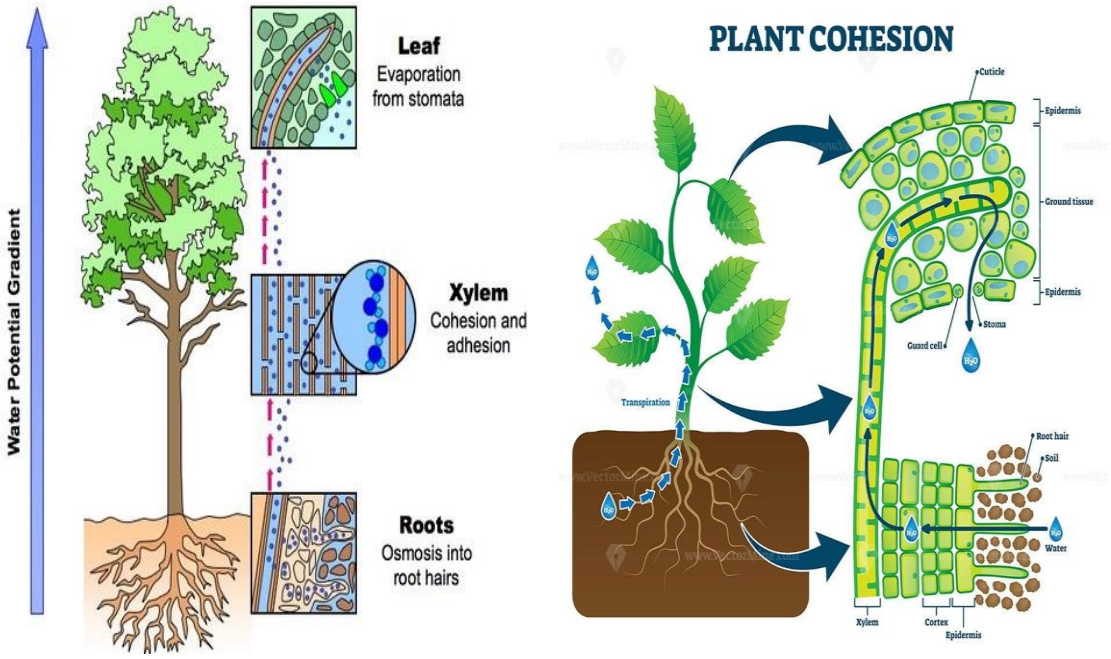
1. Atmospheric pressure This does not seem to be convincing because it cannot act on water present in xylem in roots, in case it is working, and then also it will not be able to raise water beyond 34.

2. Imbibition Sachs (1878) supported the view that ascent of sap could take place by imbibition through the walls of xylem.

D. Transpiration pull and cohesion of water theory This theory was originally proposed by Dixon and Jolly (1894) later supported and elaborated by Dixon (1924).



This theory is very convincing and has now been widely supported by many workers. Although H- bond is very weak but they are present in enormous numbers as in case of water, a very strong mutual force of attraction or **cohesive force** develops between water molecules and hence they remain in the form of a continuous water column in the xylem.

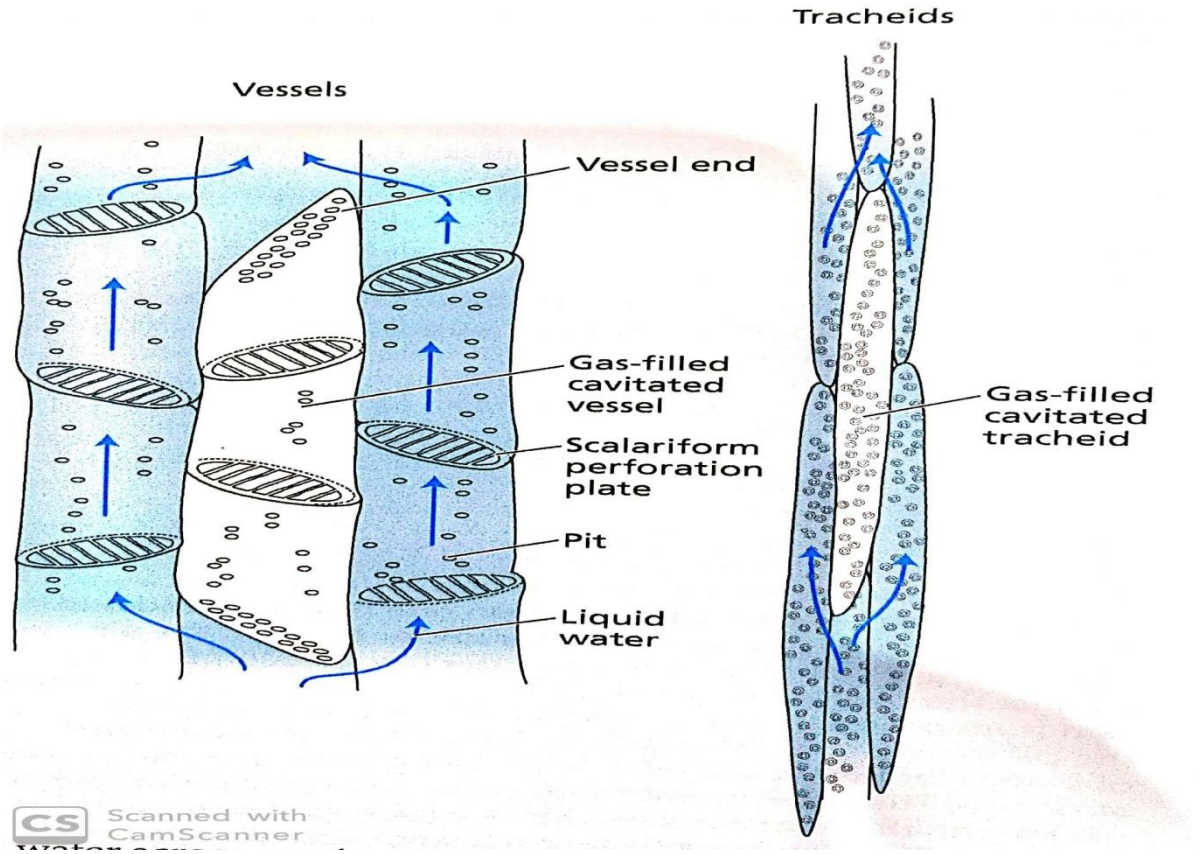


The magnitude of this force is very high, therefore the continuous water column in the xylem cannot be broken easily due to the force of gravity or other abstractions offered by the internal tissues in the upward movement of water.

The **adhesive** properties of water i.e. attractions between the water molecules and the containers walls (here the walls of xylem) further ensure the continuity of water column in xylem.

When transpiration takes place in the leaves at the upper parts of the plant, water evaporates from the intercellular spaces of the leaves to the outer atmosphere through stomata. More water is released into the intercellular spaces from mesophyll cells. In turn, the mesophyll cells draw water from the xylem of the leaf.

Due to all this, a tension is created in the xylem elements of the leaves. This tension is transmitted downward to water in xylem elements of the root through the xylem of petiole and stem and the water is pulled upward in the form of continuous unbroken water column to reach the transpiring surfaces up to the top of the plant.



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4. Internet.

Salient features of bacteriophages Bacteriophages

- **Bacteriophages**, or phages are viruses that specifically **infect bacteria**.
- Phages are very simple in structure, consisting merely of a **DNA** (or occasionally ribonucleic acid (RNA)) molecule carrying a number of genes, including several for replication of the phage, surrounded by a **protective coat or capsid** made up of protein molecules.

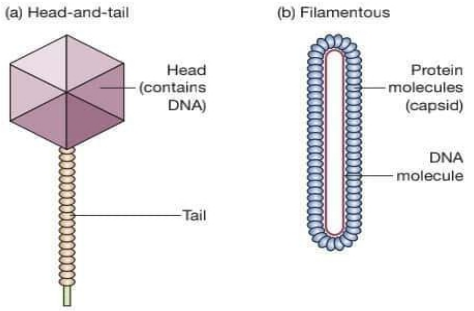


Figure 2.5
The two main types of phage structure: (a) head-and-tail (e.g. λ); (b) filamentous (e.g. M13).

The phage infection cycle

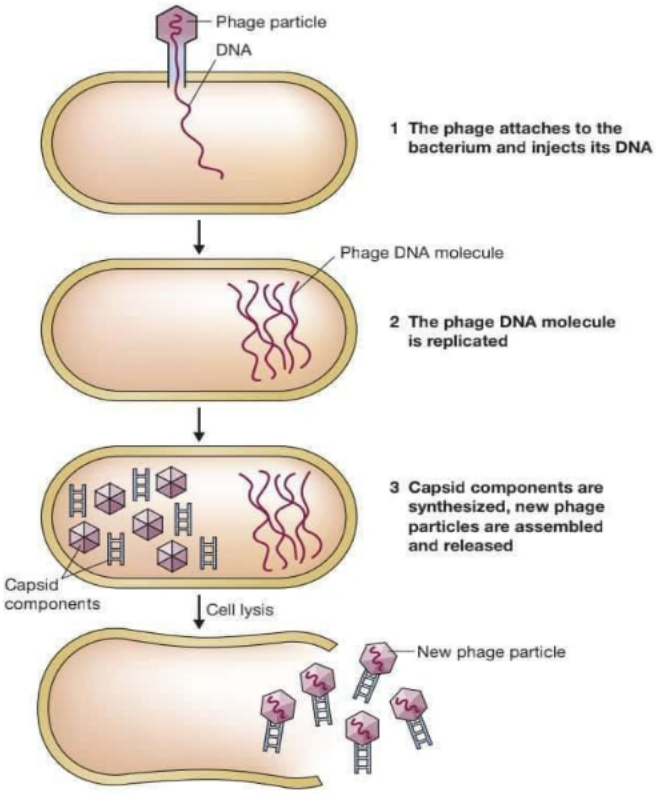


Figure 2.6
The general pattern of infection of a bacterial cell by a bacteriophage.

- The general pattern of infection, which is the same for all types of phage, is a **three-step process** (Figure 2.6):

- The phage particle attaches to the outside of the bacterium and injects its DNA chromosome into the cell.
- The phage DNA molecule is replicated, usually by specific phage enzymes coded by genes in the phage chromosome.
- Other phage genes direct synthesis of the protein components of the capsid, and new phage particles are assembled and released from the bacterium.

Lytic phages or Lytic cycle

- In some phage types the entire **infection cycle is completed very quickly**, possibly in less than 20 minutes. This type of rapid infection is called a **lytic cycle**.
- The release of the new phage particles is associated with **lysis of the bacterial cell**.
- The characteristic feature of a lytic infection cycle is that phage DNA replication is immediately followed by synthesis of capsid proteins, and the phage DNA molecule is never maintained in a stable condition in the host cell.

Lysogenic phages

- Lysogenic infection is characterized by **retention of the phage DNA** molecule in the host bacterium.
- This retention is possibly for many thousands of cell divisions.
- In many lysogenic phages the phage DNA is inserted into the bacterial genome, in a manner **similar to episomal insertion**.
- The integrated form of the phage DNA (called the **prophage**) is **quiescent**, and a bacterium (referred to as a **lysogen**) that carries a prophage is usually physiologically indistinguishable from an uninfected cell.
- However, the prophage is eventually released from the host

genome and the phage reverts to the lytic mode and lyses the cell.

- The infection cycle of lambda (E), a typical lysogenic phage of this type, is shown in Figure 2.7.

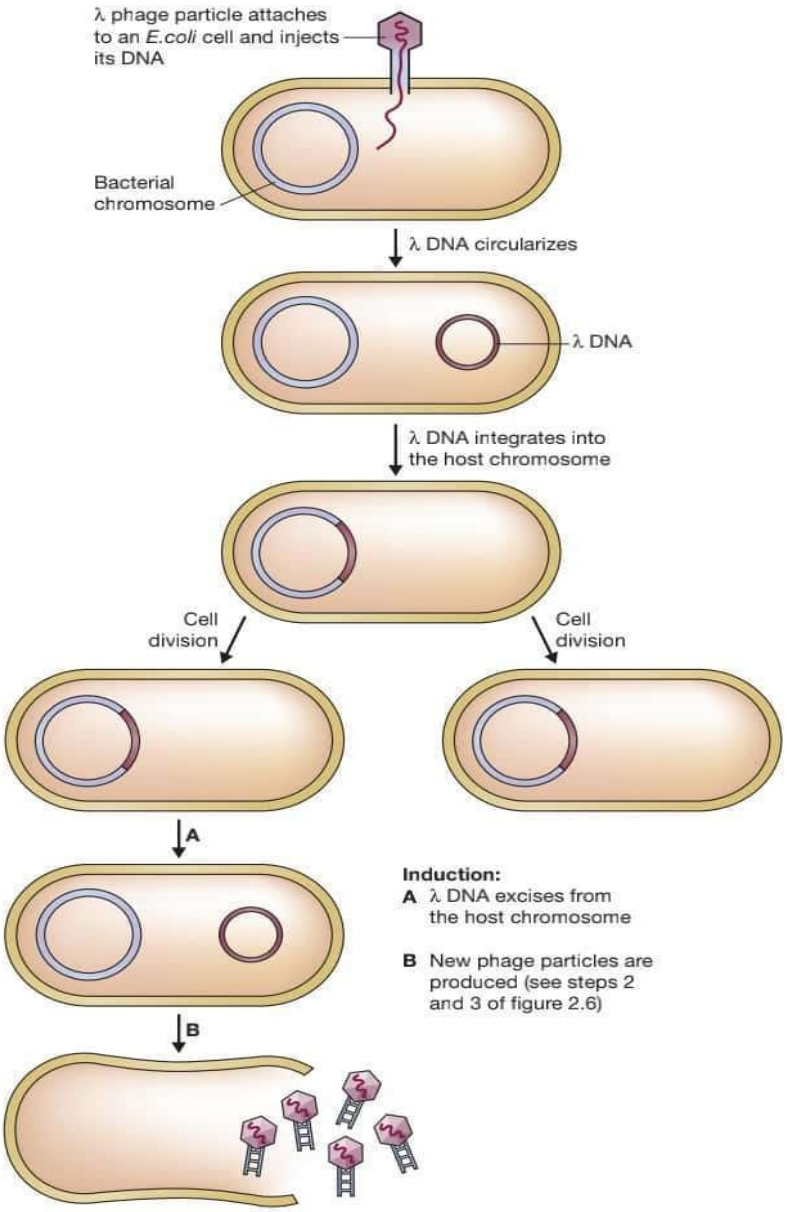


Figure 2.7

The lysogenic infection cycle of bacteriophage λ.

C_4 Cycle

or

Hatch and Slack Pathway

In C_4 cycle, the first formed stable compound is a 4 carbon compound viz., oxaloacetic acid. Hence it is called **C4 cycle.**

The path way is also called as Hatch and Slack as they worked out the pathway in 1966 and it is also called as **C_4 Dicarboxylic Acid Pathway.**

This pathway is commonly seen in many grasses, sugar cane, maize, sorghum and amaranthus. The C_4 plants show a different type of leaf anatomy.

The chloroplasts are dimorphic in nature. In the leaves of these plants, the vascular bundles are surrounded by bundle sheath of larger parenchymatous cells.

These bundle sheath cells have chloroplasts. These chloroplasts of bundle sheath are larger, lack grana and contain starch grains. The chloroplasts in mesophyll cells are smaller and always contain grana. This peculiar anatomy of leaves of C4 plants is called **Kranz anatomy.** The bundle sheath cells are bigger and look like a ring or wreath.

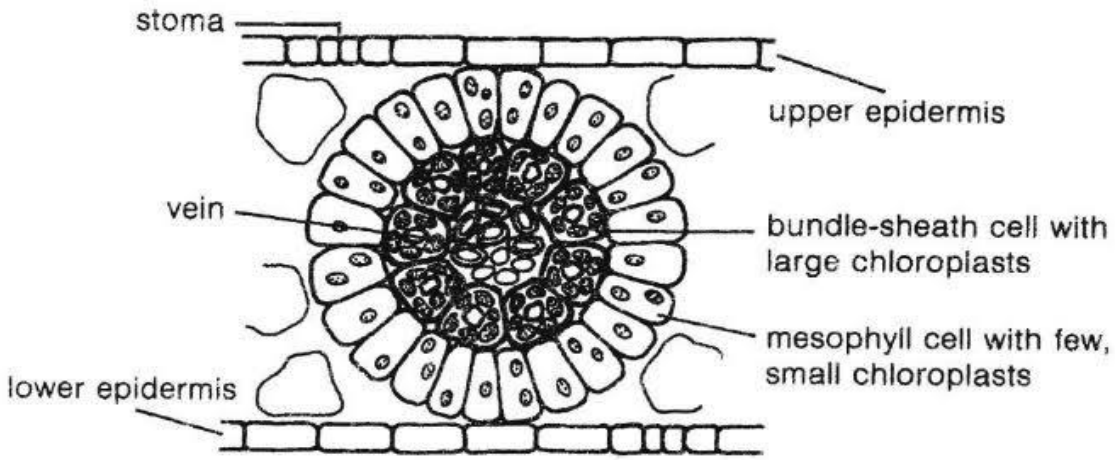
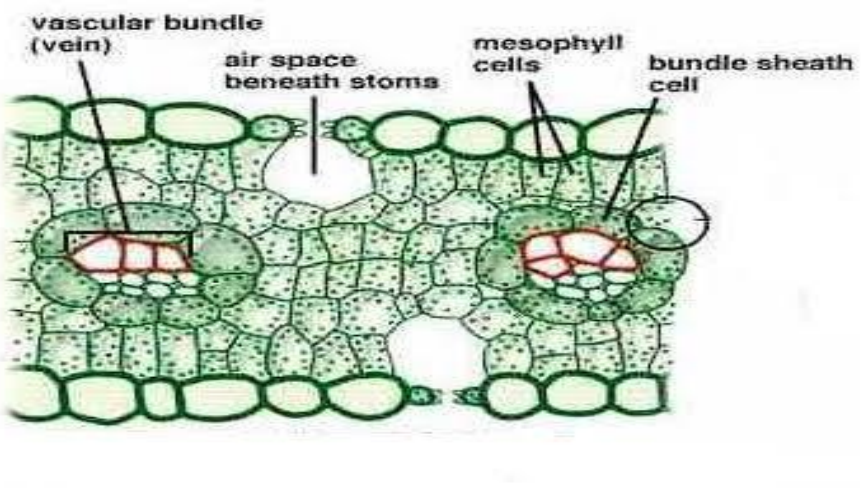


Fig 1. KRANZ ANATOMY



Kranz in German means wreath and hence it is called **Kranz anatomy.** The C_4 cycle involves two carboxylation reactions, one taking place in chloroplasts of mesophyll cells and another in chloroplasts of bundle sheath cells.

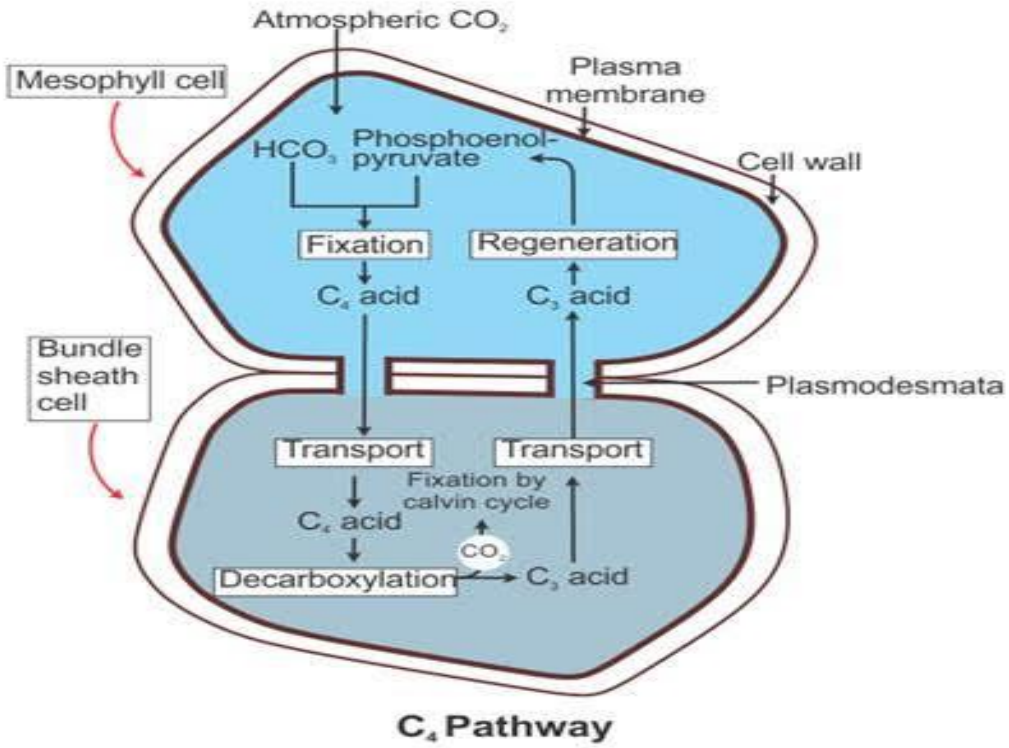
There are four steps in Hatch and Slack cycle:

1. Carboxylation

2. Breakdown

3. Splitting

4. Phosphorylation



C_4 Pathway

1. Carboxylation

It takes place in the chloroplasts of mesophyll cells. Phosphoenolpyruvate, a 3 carbon compound picks up CO2 and changes into 4 carbon oxaloacetate in the presence of water. This reaction is catalysed by the enzyme, phosphoenol pyruvate carboxylase

2. Breakdown

Oxaloacetate breaks down readily into 4 carbon malate and aspartate in the presence of the enzyme, transaminase and malate dehydrogenase. These compounds diffuse from the mesophyll cells into sheath cells.

3. Splitting

In the sheath cells, malate and aspartate split enzymatically to yield free CO_2 and 3 carbon pyruvate. The CO_2 is used in Calvin's cycle in the sheath cell

The second Carboxylation occurs in the chloroplast of bundle sheath cells. The CO_2 is accepted by 5 carbon compound ribulose diphosphate in the presence of the enzyme, carboxy dismutase and ultimately yields 3 phosphoglyceric acid. Some of the 3 phosphoglyceric acid is utilized in the formation of sugars and the rest regenerate ribulose diphosphate

4. Phosphorylation

The pyruvate molecule is transferred to chloroplasts of mesophyll cells where, it is phosphorylated to regenerate phosphoenol pyruvate in the presence of ATP. This reaction is catalysed by pyruvate phosphokinase and the phophoenol pyruvate is regenerated.

In Hatch and Slack pathway, the C_3 and C_4 cycles of carboxylation are linked and this is due to the Kranz anatomy of the leaves. The C_4 plants are more efficient in photosynthesis than the C_3 plants. The enzyme, phosphoenol pyruvate carboxylase of the C_4 cycle is found to have more affinity for CO_2 than the ribulose diphosphate carboxylase of the C_3 cycle in fixing the molecular CO_2 in organic compound during Carboxylation.

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Crassulacean Acid Metabolism (CAM) Cycle

OR

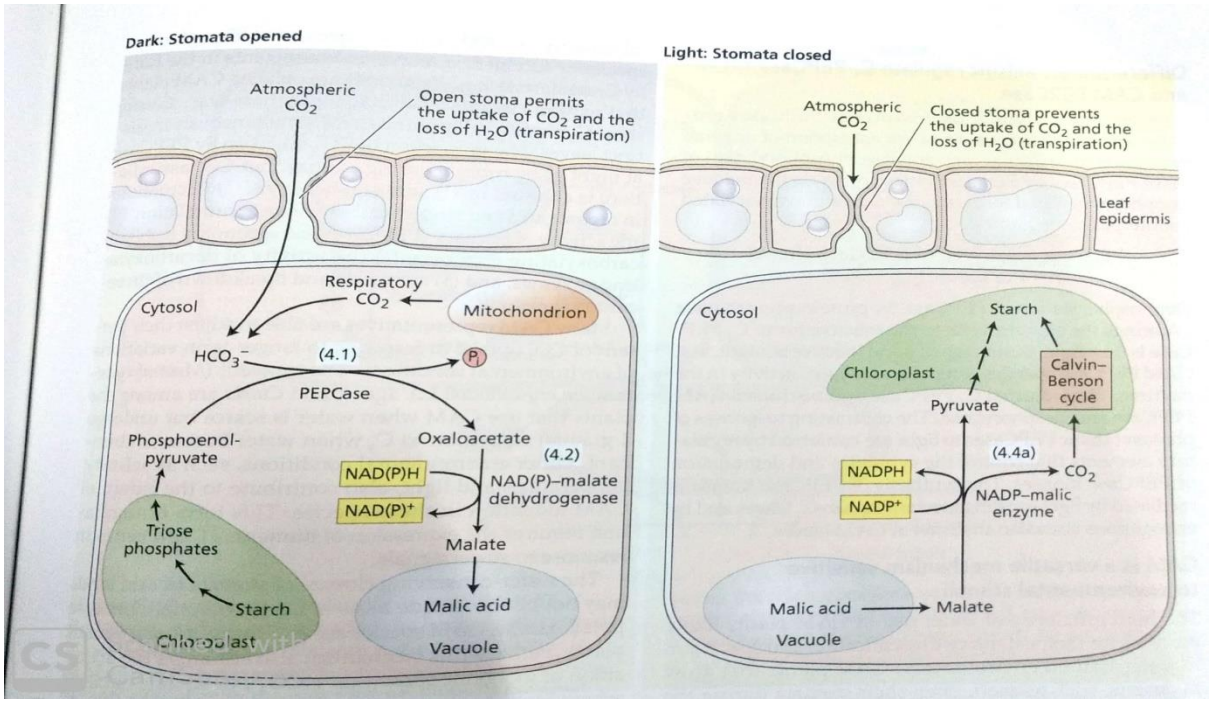
The Dark Fixation of CO_2 in Succulents

CAM is a cyclic reaction occurring in the dark phase of photosynthesis in the plants of Crassulaceae. It is a CO_2 fixation process wherein, the first product is malic acid. It is the third alternate pathway of Calvin cycle, occurring in mesophyll cells. The plants exhibiting CAM cycle are called **CAM plants**.

Most of the CAM plants are succulents e.g., Bryophyllum, Kalanchoe, Crassula, Sedium, Kleinia etc. It is also seen in certain plants of Cactus e.g. Opuntia, Orchid and Pine apple families.

CAM plants are usually succulents and they grow under extremely xeric conditions. In these plants, the leaves are succulent or fleshy. The mesophyll cells have larger number of chloroplasts and the vascular bundles are not surrounded by well defined bundle sheath cells.

In these plants, the stomata remain open during night and closed during day time. The CAM plants are adapted to photosynthesis and survival under adverse xeric conditions. CAM plants are not as efficient as C_4 plants in photosynthesis. But they are better suited to conditions of extreme desiccation.



CAM involves two steps:

1. Acidification 2. Deacidification

1. Acidification

In darkness, the stored carbohydrates are converted into phophoenol pyruvic acid by the process of Glycolysis. The stomata in CAM plants are **open in dark** and they allow free diffusion of CO_2 from the atmosphere into the leaf. Now, the phosphoenolpyruvic acid carboxylated by the enzyme phosphoenol pyruvic acid carboxylase and is converted in to oxalaoacetic acid.

The oxaloacetic acid is then reduced to malic acid in the presence of the enzyme malic dehydrogenase. The reaction requires $NADPH_2$ produced in Glycolysis.

The malic acid produced in dark is stored in the vacuole. The malic acid increases the acidity of the tissues.

2. Deacidification During day time, when the stomata are closed, the malic acid is decarboxylated to produce pyruvic acid and evolve carbon dioxide in the presence of the malic enzyme. When the malic acid is removed, the acidity decreases the cells. This is called **deacidification.** One molecule of $NADP^+$ is reduced in this reaction.

The pyruvic acid may be oxidized to CO_2 by the pathway of Kreb's cycle or it may be reconverted to phosphoenol pyruvic acid and synthesize sugar by C_3 cycle. The CO_2 released by deacidification of malic acid is accepted by ribulose diphosphate and is fixed to carbohydrate by C_3 cycle. CAM is a most significant pathway in succulent plants. The stomata are closed during day time to avoid transpiration loss of water.

As the stomata are closed, CO_2 cannot enter into the leaves from the atmosphere. However, they can carry out photosynthesis during the day time with the help of CO_2 released from organic acids.

During night time, organic acids are synthesized in plenty with the help of CO_2 released in respiration and the CO_2 entering from the atmosphere through the open stomata. Thus, the CO_2 in dark acts as survival value to these plants

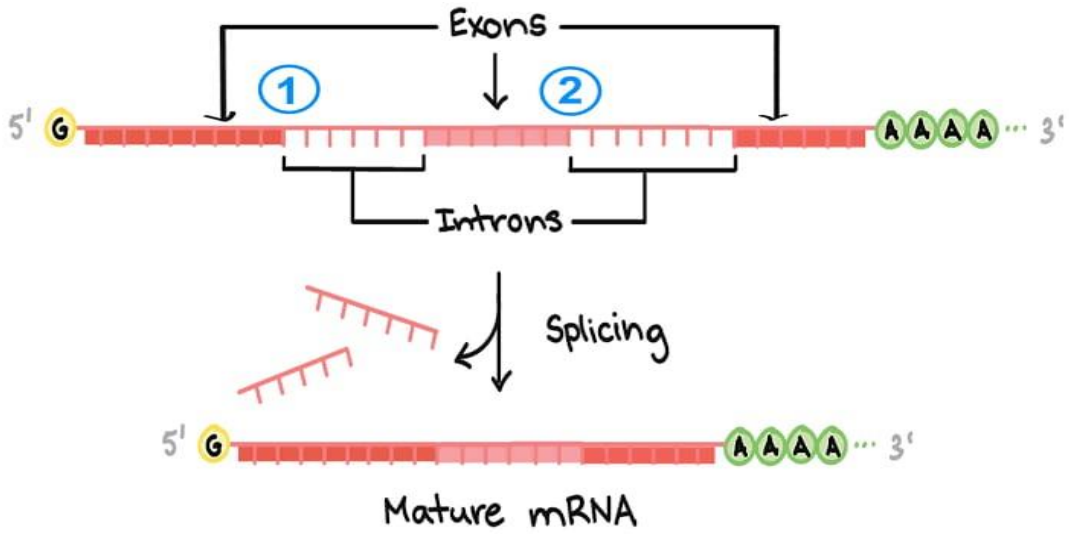
c- DNA Library

A c-DNA library is a collection of cloned DNA sequences that are complementary to the mRNA extracted from an organism or tissue of interest (c- stands for complementary).

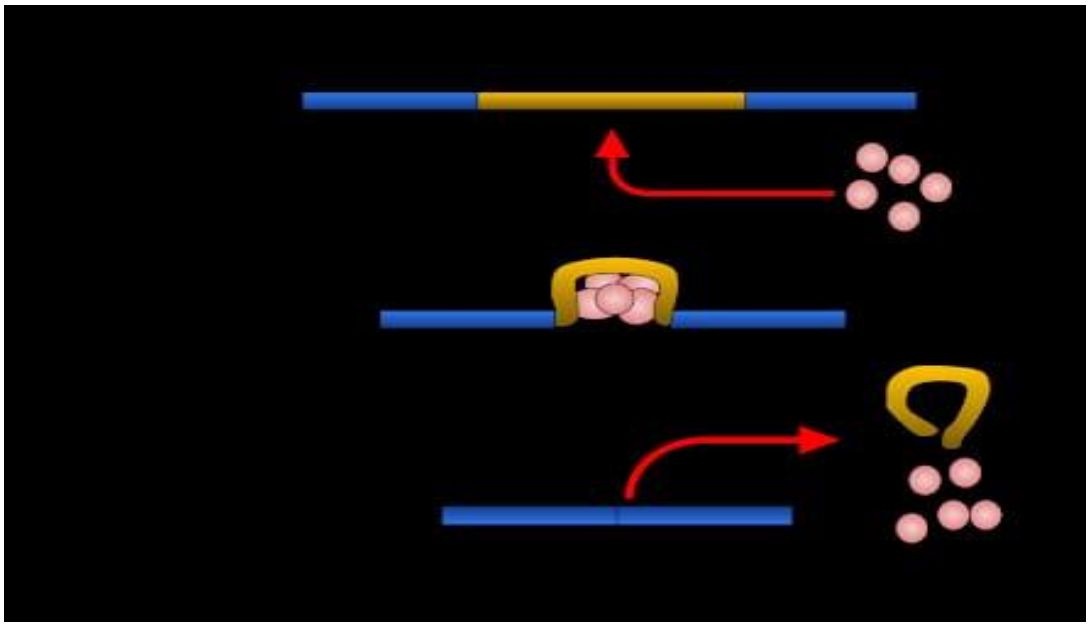
c-DNA is produced from fully transcribed mRNA i.e. from mature mRNA found in the nucleus and therefore contains only the expressed genes of an organism.

mRNA is spliced before translation into protein in eukaryotic cells, hence the DNA synthesized from spliced mRNA does not have non-coding regions or introns of the gene.

How splicing takes place is depicted in next two figures.

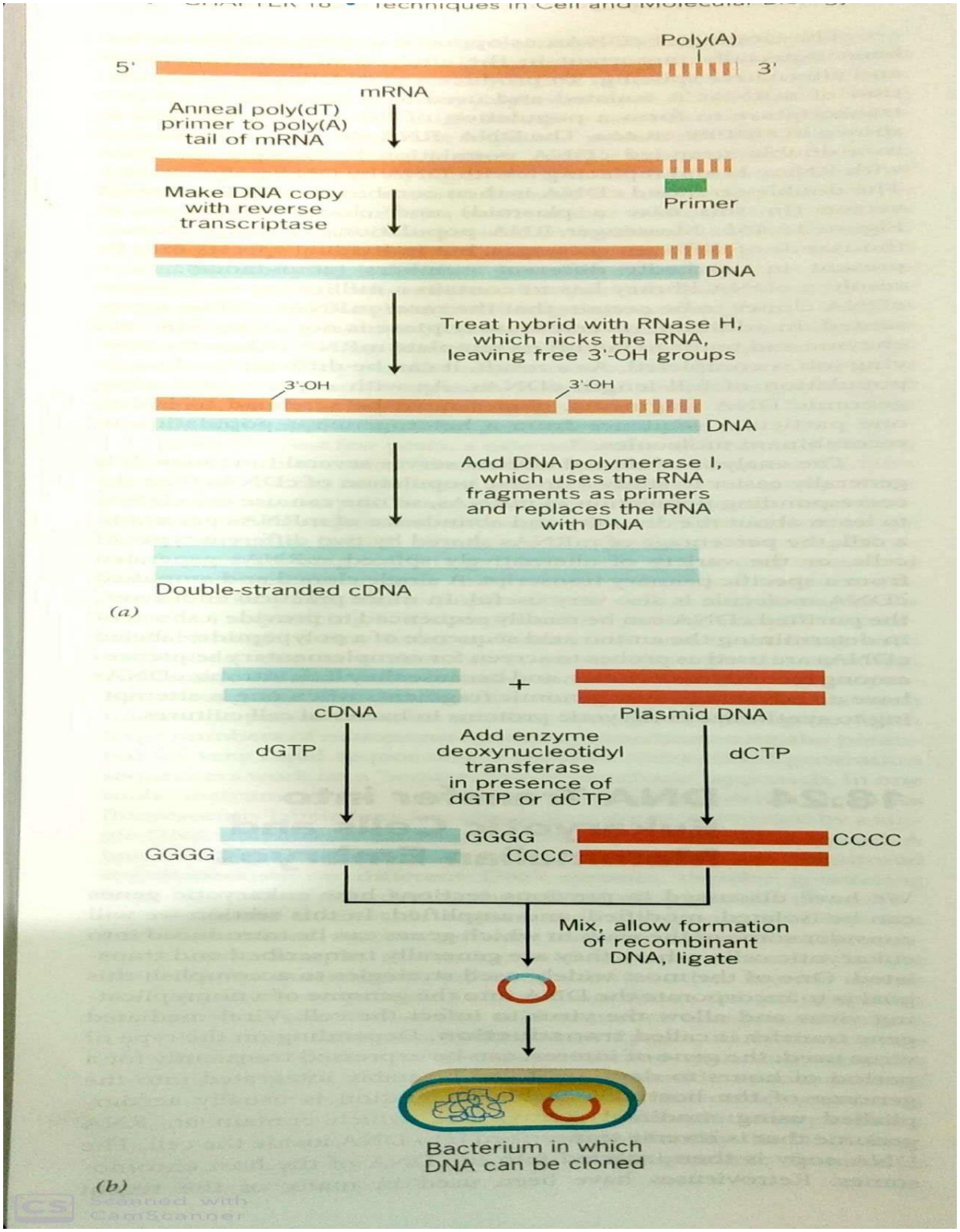


Splicing takes place with the help of a complex called spliceosome made up of (snRNPs) small nuclear ribonucleoproteins.



1. A group of 5 snRNPs are needed to bind to the intron of pre-mRNA and remove it to leave only the exons.
2. The snRNPs bind to the intron and cause it to fold to bring the ends of intron closer forming a loop and ends of exon move closer to join together.
3. Intron detaches and exons join to form mature mRNA. snRNPs also detach and are used for more splicing reaction.

- Construction of c-DNA library first of all involves the isolation of total mRNA from a cell type or tissue of interest.
- The 3' ends of eukaryotic mRNA are composed of a string of 50-250 adenylate residues (poly A tail) which makes the separation easy from the much more abundant rRNA and t-RNA in a cell extract by using a column containing oligo-dT's tagged onto its matrix.
- So a population of mRNA is isolated, mRNA molecules contain 3' poly A tails.
- Poly T oligomers can be used as primers for the synthesis of c-DNA strands by reverse transcriptase to form a population of DNA-RNA hybrids.
- Then the RNA-DNA duplex are converted to ds Dna molecules by combined activities of ribonuclease H, DNA polymerase I and DNA ligase.
- Ribonuclease H degrades the RNA template strand by nicking it.
- Short RNA fragments produced during degradation serve as primers for DNA synthesis
- DNA polymerase I catalysis the synthesis of second DNA strand and replaces RNA primers with DNA strands.
- DNA ligase seals the remaining single stranded breaks in ds DNA molecule.
- These ds c-DNA's can be inserted into plasmid or phage lambda cloning vectors by adding complementary single stranded tails to the c-DNAs and vectors.
- To prepare blunt ended c-DNA for cloning, a short stretch of poly G is added to the 3' ends of the c-DNA and a complementary stretch of poly C is added to the 3' ends of the plasmid DNA.
- The two DNAs are mixed and allowed to form recombinants, which are sealed and used to transform bacterial cells in which they are cloned.



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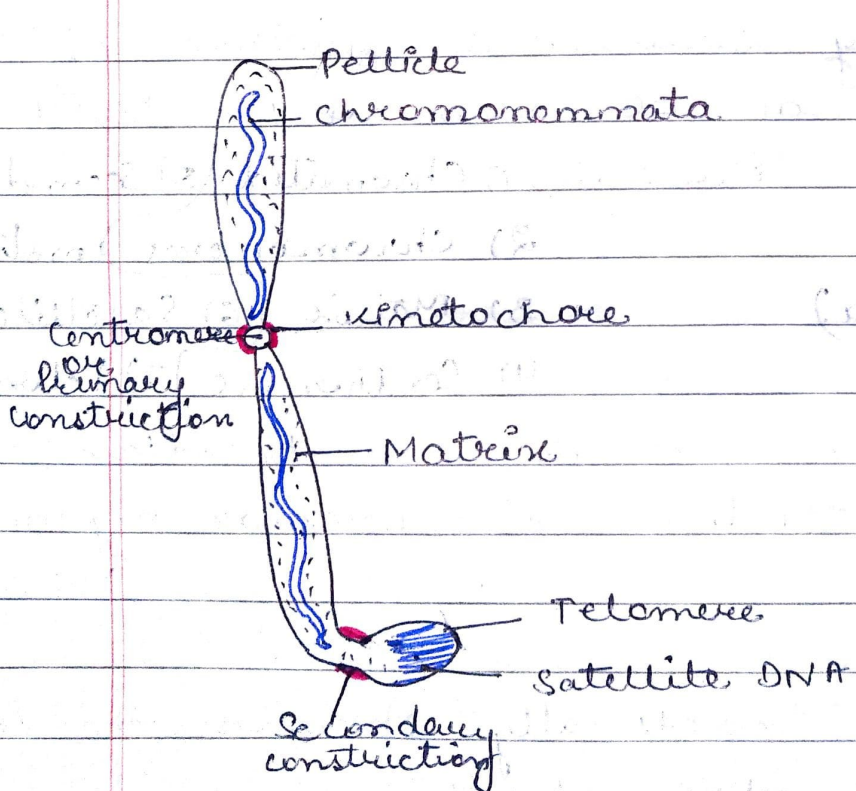
CHROMOSOME – Chromosome are the rod-shaped dark stained bodies seen during the metaphase stage of mitosis. Chromosome were first described by Strasburger in 1815 and term chromosome was coined by Waldeyer in 1888.

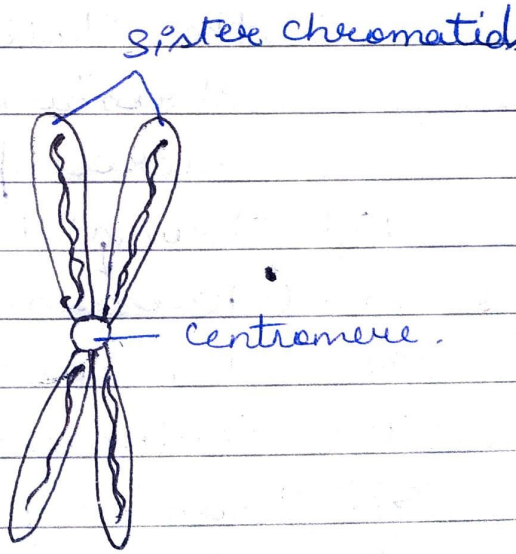
OR

Chromosome = DNA + protein (Histone) = Chromatin

↓

Histone proteins are defined as group of small proteins that contain high content of basic amino acids such as arginine and lysine. DNA and histones are organised into repetiting subunits called as nucleosomes.





Diploid : 2 set of chro...

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Chromosome Number - The no. of chromosome is constant for a particular species. Therefore, these are of great importance in the determination of phylogeny and taxonomy of the species. The number of set of the chromosome of the gametic cells such as sperm and ova ('gametic or reduced or haploid set of chromosomes). The haploid set of the chromosome is also known as genome.

Morphology - Chromosome morphology changes with the stage of cell division and the most suitable stage for chromosome study are metaphase. In metaphase stage, the following structural features of chromosome can be seen under light microscope :- (Physical)

1) Chromatid
2) (Chromonemma)
3) Chromomeres
4) Centromere or Primary constriction,
5) Secondary constriction or nucleolar organiser.
6) Telomere
7) Satellite DNA.

1) Chromatid. 5) Secondary
2) Chromomeres constriction
3) Matrix 6) Satellite
4) Centromere 7) Telomere

Structurally chromosome is devided into three parts :

1) Pellicle
2) Matrix
3) Chromonemmata.

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1) **Pellicle** - It is the outer covering around the chromosome. It is very thin layer.

2) **Matrix** - It is the ground substance of chromosome which contain chromonemmata.

3) **Chromonemmata** - It is embedded in the matrix of each chromosome. There are two identical thread like spirally coiled (form double helical structure of DNA)

1) **Chromatid** - At metaphase stage, each chromosome consists of two symmetrical structure called chromatid. Each chromatid contain a single DNA molecule. Both the chromatids are attached with each other by centromere and they separated at the begining of anaphase stage.

2) **Centromere** - Centromere is a part of chromosome is recognised as permanent and it is the small structure in the chromonemma and is marked by constriction. At this point, the two chromonemmata are joined togethere. This is also known as primary constriction.

Kinetochore - A kinetochore is a disc shaped proteinous structure where the spindle fibres attached during cell division to pull the chromatid apart. Its proteins also help to hold the sister chromatid togethere and play a role in chromosome editing.

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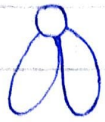
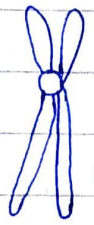
Depending upon the location of the centromere the chromosome are divided into following:

1) **Telocentric**- are rod shaped chromosome in which centromere occupying the terminal position so that the chromosome has just one arm.

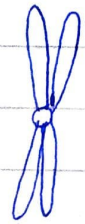


2) **Acrocentric**- are also rod shaped chromosome with centromere occupying a subterminal position in which one arm is very long and other is very short.

3) **Submetacentric**- in which centromere are slightly away from the mid point, arms are unequal.



4) **Metacentric** – (V-shaped) - in which the centromere lies in the middle of the chromosome so that two arms are almost equal in length.



Difference b/w chromosome and chromatin.

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3) **Secondary constriction** – Secondary constriction can be distinguish from primary constriction or centromere because of chromosome bends at the position of anaphase. The region between secondary constriction and telomere is called as **satellite**. Therefore chromosomes having secondary constriction are called **satellite DNA** or **sat chromosome**. The number of sat chromosome in genome varies from one species to other. Nucleolus is always linked with secondary constriction of sat chromosome. Therefore secondary constriction are also called as **Nucleolus Organisee Region** (NOR). This region contains several hundreds ~~hobbies~~ copies of genes coding for ribosomal RNA.

4) **Telomere** – These are specialised ends of chromosome or terminal end of chromosome or chromosomal end known as **telomere**.

Chemical composition of chromosome – Chemically chromosomes are composed of DNA, proteins, RNA. The proteins of chromosome are of two types: 1) Histone protein
2) Non-histone protein.

(30–40 % DNA, 50–65 % Protein, 0.3–10 % RNA)

It is the major constituent of chromosomes that store genetic information in coded form.

1) **DNA** – The amount of DNA present in normal somatic cells of species is constant, any variation in DNA is strictly correlated with variation in the chromosome.

RNA — Most of the RNA transcribed by DNA, migrate to the nucleolus & cytoplasm. A small portion of it remain attached with DNA molecule along with protein.

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Protein - Proteins are of two types:

1) Histone
2) Non- histone

1) Histone - Histones are basic protein and they are enriched with basic amino acids such as arginine and lysine. The histone proteins are of five types:-

1) H_1
2) H_2A
3) H_2B
4) H_3
5) H_4

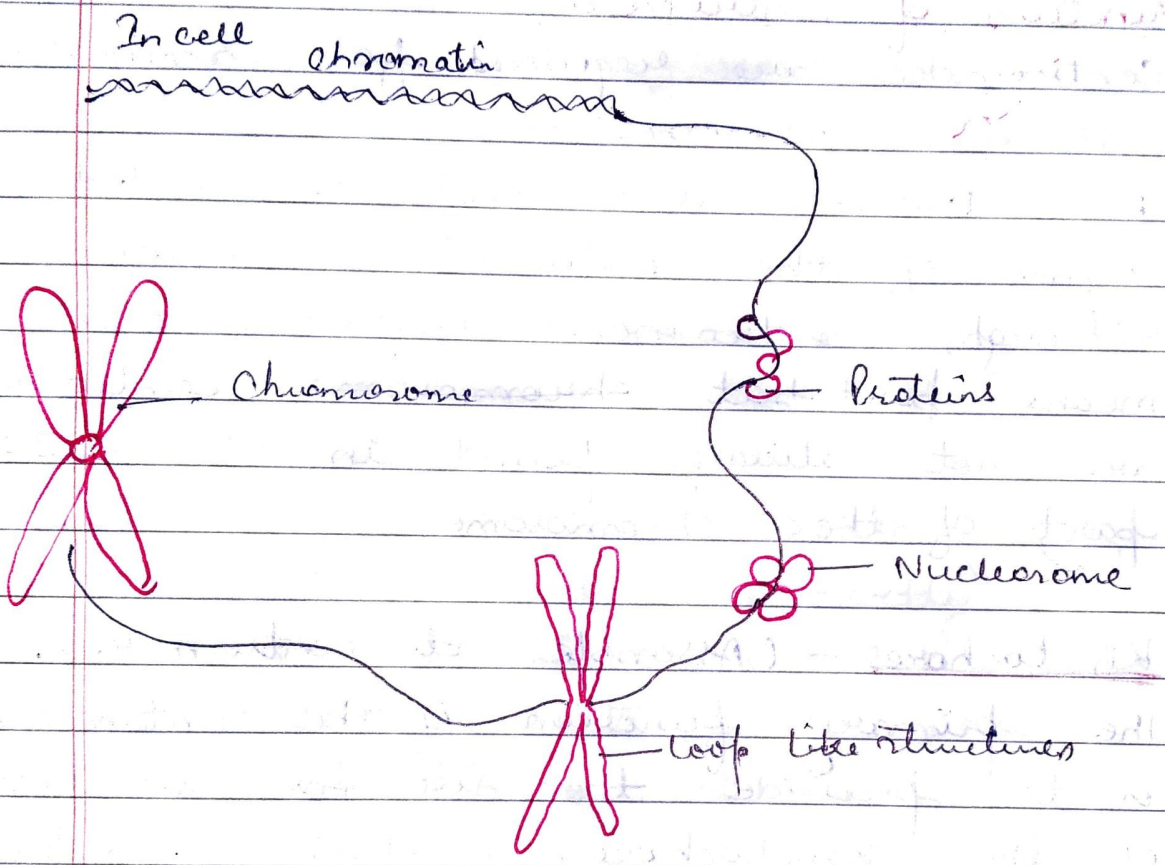
H_1 is loosely bounded with DNA. Histone plays a primary function in chromosome organisation where H_2A, H_2B, H_3 and H_4 are involved in structural organisation of the chromatin while H_1 hold together the folded chromatin fibres of chromosome.

Non- histone make 20% of total chromosomal mass. Three major types of non- histone proteins -
1) Structural 2) Enzymatic 3) Regulatory.

Functions of Chromosome - 1) The chromosome play a very important role in hereditary.
2) It is universally accepted that DNA is the genetic material while RNA and in eukaryotes almost all the DNA is present in chromosome.
3) The most important function of chromosome is to provide genetic information from various cellular organs.

4) Another important function of chromosome is to protect the genetic material from damage (cell division).

5) Centromere of chromosome perform an important function in chromosomal movement.



Centromere - Without centromere cells cannot divide properly and therefore overall process of mitosis donot perform. Mitosis is the process by which eukaryotic cells divides and reproduce two daughter cells that each contain the same no. of chromosome as the parent cell. The centromere was first described by German biologist Walter Flemming in 1880 as the primary constriction of the chromosome. The

centromere is a region of specialised chromatin that provides site for sister chromatid attachment. If any error occur in the centromere or kinetochore function leads to cancerous growth.

Function of centromere

1) Centromere are required for accurate site of chromosome.
2) A centromere is a most constricted region of the condensed chromosome. Although, centro means central, mere means part but ~~chromosome~~ centromere are not always found in the central part of the chromosome.

2) **Kinetochores** – (Assemble at centromere). The primary function of the centromere is to provide the site for attachment of the kinetochore. Kinetochore is the proteinous complex structure and it is required for proper chromosomal saggregation during mitosis. In electron micrograph mitotic chromosome, it appears as plate like or disc like structure composed of several layers.

3) **Another function** – Sister Chromatids are joined together at centromere. In addition to their centromere perform another

essential feature in mitosis by providing as the site of sister chromatid cohesion/ attachment.

Telomere - Telomere are the regions of DNA at the end of eukaryotic chromosome that are required for replication and stability of the chromosome. Telomeric ends in case of vertebrates form caps at their ends so that they protect the chromosome from nuclease and from destabilisation. They prevent the end of chromosome from fusing with one another. Human telomere contain the sequence (TTAGGG) repeted from about 500 - 5000. Shrinkage of telomere causes chromosome shortly and therefore cells stop to grow and divide.

Telomeric replication - It is an important aspect of DNA replication.

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Sex Chromosome - Sex chromosome is also known as hetero chromosome or Idio chromosome or allosomes is the sex chromosome that differ from an ordinary autosome in form, size and behaviour. The human sex chromosome are typical pair that determine the sex of an individual created by a sexual reproduction. In human each cell nucleus contain 23 pairs of chromosome total of 46 chromosome. The first 22 pairs are called autosomes. They are homologous chromosome (chromosome which contain same genes or DNA in the same order along their chromosomal arms) The Chromosome of 23 paired are called sex chromosome are called allosomes consisting of two X chromosome in most females and one X and one Y chromosome in most of the males. Females therefore have 23 homologous chromosomal pair and while in male have 22 allosomes.

23 pairs.

- 22 ~~Atto~~ pairs
  Autosomes
- 1 pair
  Sex chromosome or allosomes
  - Male
    XY
  - Female
    XX

Wheat: Origin and Cultivation

Botanical Name: *Triticum aestivum* L.. (X=7) Family: *Poaceae*

Wheat belongs to the tribe Triticeae (= Hordeae) in the grass family Poaceae (Gramineae)

Wheat is world's most widely cultivated food crop and in India it is the second important staple cereal food. India is the second largest producer of wheat after China. Uttar Pradesh, Punjab, Haryana, West Bengal, Bihar etc. are major wheat growing states in India. Duration of wheat crop is 110-130 days.

Origin of Wheat

Wheat is cultivated since pre-historic times in the world. From all possible records, it seems that its center of origin is South Western Asia. It is believed that Aryans brought wheat grains to India, and since then it is being grown in India. Records from ancient China show that it was raised there since 2700 BC, and it was also known to Egyptians and inhabitants of Switzerland as early as stone age. The centres of origin of *Triticum* species are given below.

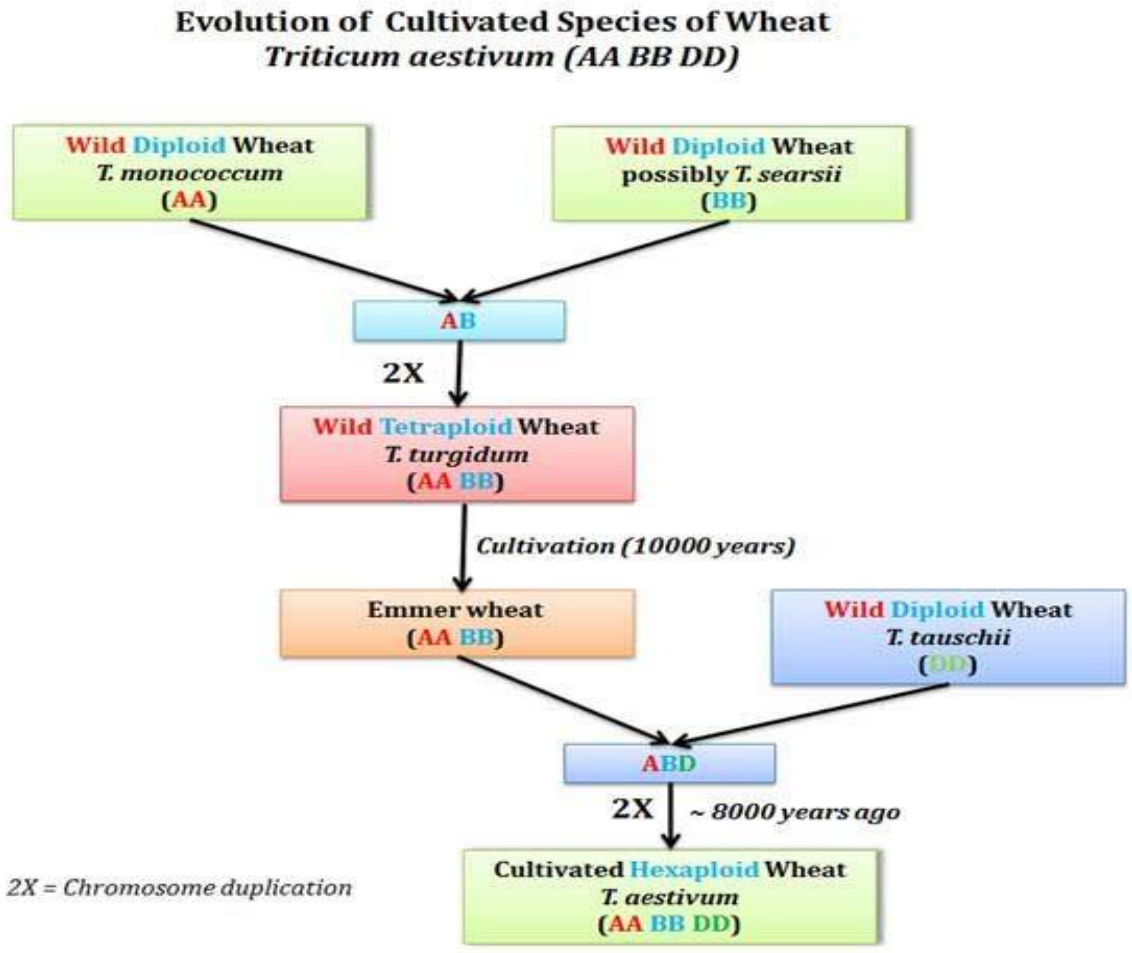
Centres of origin of *Triticum* species

Species (Ploidy level)	Common name	Centre of origin
T. aestivum (6x)	Bread wheat	Central Asia, North East

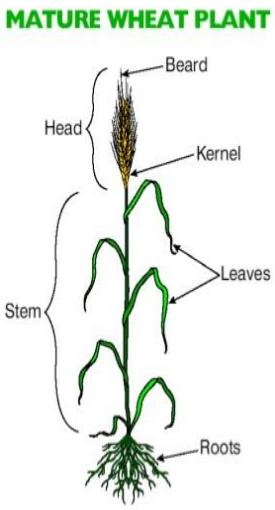
T. dicoccum (6x)	Emmer wheat	Abyssinia
T. durum (4x)	Macaroni wheat	Near East, Mediterranean region, Abyssinia
T. turgidum (4x)	Rivet or cone wheat	
T. monococcum (4x)	Einkorn wheat	Near East
T. compactum (6x)	Club wheat	Central Asia
T. sphaerococcum (6x)	Short wheat	Central Asia

Source: Zeven and Zhukovsky (1975) and Hawkes (1982)

Evolution of Bread Wheat



Botanical Description of Wheat



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Wheat plant can be divided in 2 distinct parts, viz., root system and shoot system.

1. **Root system:** As the wheat plants belong to monocot family, roots are adventitious in nature.

2. **Shoot system**: Visible part above the ground. It comprises of stems, leaves and inflorescence. A stem and inflorescence may be called a **'*culm*'**.

    - ***Stem****:* The stem of wheat plant is erect, cylindrical, jointed and smooth. In bread wheat, the stems are hollow, except at the nodes where they are solid, but in a few cultivars of Macaroni wheat, the internodes are completely filled with soft pith.
    - *Leaves:* The wheat leaves consists of the following 4 parts. *Leaf sheath*: The basal part of the leaf, which encircles the stem and the blade that bends away from the stem. *Leaf blade*: the flattened, parallel veined portion of the leaf. *Ligule*: the membranous outgrowth is called ligule. *Auricle*: lobes of the leaf blade, which extend downward on each side at the junction of the blade and sheath. These are claw-like appendages projecting from the collar of the leaf.
    - *Inflorescence:* The inflorescence is known as '*ear*' or '*head*', but its botanical name is spike. Spikelets are systematically arranged and are distributed along the central zig-zag axis '*rachis*'. The spikelets are borne on alternate sides of

the rachis, which gives it a zig-zag appearance.

- *Spikelet*: It is composed of flowers called florets. The number of florets in a spikelet may vary from 1 to 5. The florets in each spikelet are enclosed by 2 glumes.
- *Kernel:* Wheat has a **'*caryopsis*'** type of fruit. The typical wheat kernel is 3 to 10 mm in length and 3 to 5 mm in diameter.

Various Growth Stages of Wheat Plant

Wheat plant passes through various stages of growth, as described below:

Pre-establishment stages

1. *Pre-emergence:* Germination of seeds, which produce seminal roots and coleoptiles.
2. *Emergence:* Germinating seeds produce coleoptiles above the soil surface.

Vegetative stages

3. *Seedling:* The young plants establish larger root systems in their seedling stage.
4. *Crown root stage:* This coincides with three or four leaf stage of plant.
5. *Tillering:* Plant produces crown & branch out into tillers from their base at soil surface
6. *Jointing:* At this stage, the plant starts elongating when the nodes start developing above the crown node.

Reproductive stages

7. *Booting:* In this stage, the upper most leaf swells out into flag holding the spike into it.
8. *Heading:* In this stage, the spike starts emerging out from the leaf sheath.
9. *Flowering:* At this stage, anthesis of florets and fertilization of ovaries take place.

Post-anthesis stages

10. *Filling:* After fertilization, the ovaries start elongating in ovules or seed passing through milk, soft-dough and hard-dough stages.
11. *Maturity*: At this stage, the colour of glumes changes and kernels become fairly hard.

Cultivation of wheat

Climate

In India, Wheat is grown as Rabi (winter) crop. The optimum temperature range for ideal germination of wheat seed is 20-25°C. Temperatures above 25°C during this period tend to decrease grain weight. Wheat can be grown successfully in those regions where annual rainfall varies from 25 to 150 cm.

Soil requirement

Wheat, though grown on a wide variety of soils, prefers fertile, well-drained medium textured loamy to clay loams. A good crop of wheat can also be raised in sandy loams and black soils. The soil depth should be greater than 25 cm for wheat cultivation.

Land Preparation

After the harvest of preceding crop, the land should be ploughed..

In recent times, with the development of seed drills, *"zero tillage*" sowings are practiced after rice especially. This practice not only economizes cost of land preparation, but also reduces weed menace owing to non-ploughing of the land.

Manure and Fertilizers

Manures and fertilizers both play a very important role in wheat cultivation. Use of manure improves the general physical condition, structure of the soil and its water-holding capacity. About 10-15 tonnes of well rotten FYM or compost should be incorporated 4-6 weeks before sowing and worked well into the soil.

Time of sowing: Time of sowing has a marked influence on the yield of wheat. It depends mostly on soil temperature, irrigation facilities and duration of wheat cultivars. The normal time for sowing of high-yielding cultivars in irrigated areas begins in early November.

Seed Rate: Seed rate varies with cultivar used depending upon its seed size, moisture content in the soil, germination percentage, tillering ability, time of sowing and method of sowing. Usually, a seed rate of 100 kg/ha is sufficient under favourable conditions of normal sowing.

Spacing: For normal sown crop, a row spacing of 22.0-22.5 cm is recommended. In late sown crop, a closer spacing of 15-18 cm should be adopted.

Irrigation: The crop requires 45 cm of irrigation water, which may vary with type of soil, variety grown etc. The critical stages of irrigation:

In timely sown wheat, first irrigation is given between 20-25 days after sowing (DAS- days after sowing).

The second irrigation is applied between 40-45 DAS, which coincides with tillering stage. Third irrigation is provided at late jointing stage between 65-75 DAS.

The fourth irrigation is given at the flowering stage between 90-95 DAS.

The fifth and sixth irrigations are applied at milking stage (110-115 DAS) and dough stage (120-125 DAS) respectively.

Wheat varieties: Sonalika, Kalyansona ,Ganga, PBW-443, PBW- 343, Prasad, Naina, Halna etc.

Harvesting and threshing:

Wheat should be harvested when their leaves and stem turn yellow and become dry. To avoid loss in yield, the crop should be harvested before it is fully ripe. Harvesting in proper time ensures optimum grain quality. Harvesting is normally done by hand with the help of sickles. Now a day, power driven threshers are becoming very popular as they provide easy threshing in less time.

Utilization

It is mostly eaten in the form of *chapaties*, also consumed in various other forms such as poories, *dalia, halwa*, sweet meals etc.

In areas where rice is the staple food, wheat is used in the form of *upma* or *poories.* It is also used for manufacturing of bread, flakes, cakes, biscuits etc. Wheat contains more protein [8-15% (grain), 8-13% (flour)] than other cereals.

Wheat straw is good source of feed for livestock in our country.

Besides being nutritious, these are principally concerned with providing the characteristic substance gluten.

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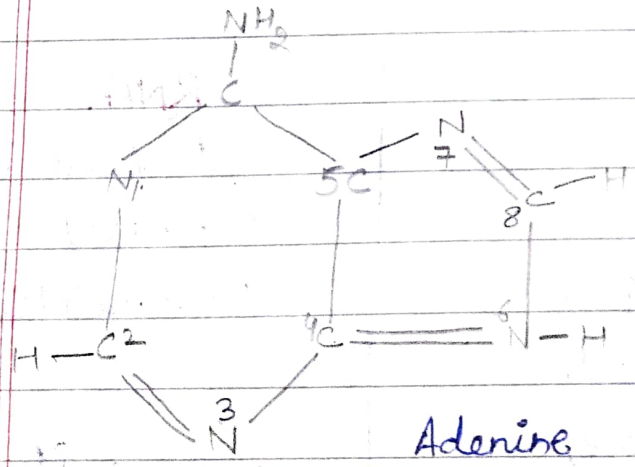
Nature of chemical Structure of DNA:-

DNA molecule is a macromolecule composed of repetiting subunits called nucleotides and each nucleotides consists of three groups: phosphate group, 5-carbon pentose group, cyclic nitrogenous containing compound. In DNA, the sugar is deoxyribosenucleic acid (In RNA sugar is ribonucelic acid). Four different nitrogenous base such as Adenine, Guanine, Thymine, Cytosine are found in DNA. In RNA, U in place of Thymine, Uracine is present. Adenine and Guanine are purines and they are double ring bases.



Adenine

Cytosine, Thymine are single ring bases and are called Pyrimidines. In polynucleotides DNA subunits are joined together in long chain

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DNA Structure / Double Helical.

The one of the most exciting breakthrough in the history of molecular biology occured in 1953, when J. Watson and F. Crick discovered the correct structure of DNA molecule. Their double helix model suggest the mechanism for the transmission of genetic characters / information. The double helical structure was based on two major evidences:

1) E. Chargaff's Rule :- when E. Chargaff and his collegues analyse the composition of DNA from different organisms and they found that the conc. of Thymine was always equal to Adenine and they Cytosine was always equal to Guanine. and their result stiongly suggest that A, G, C T were present in DNA in some fixed amount. Their data also show that the conc. of P was always equal to the conc. of G.

$$\frac{A+G}{T+C} = \frac{A+T}{G+C}$$

2) X-ray differentiation pattern :- Wilkins and Franklin and their co-workers detect the x-ray differaction of DNA molecule and they indicate that DNA was highly order two stand structure with repeating subunits along the axis of molecule. On the basis of Chargaff's rule data, Wilkin and

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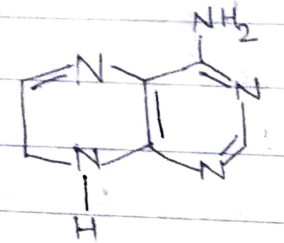
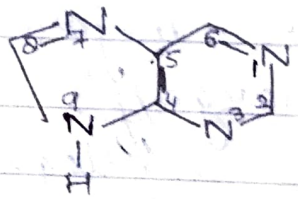
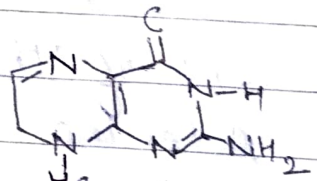
Franklin diffraction data, Watson
On the basis of these two evidences Watson and Crick proposed that DNA as the right handed double helical structure in which two polynucleotide chains are coiled with one another.



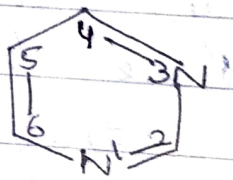
Adenine



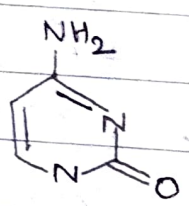
Purine



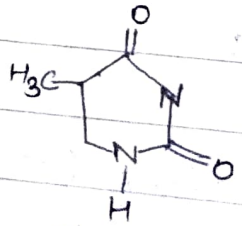
Guanine



Pyrimidine.



Cytosine



Thymine

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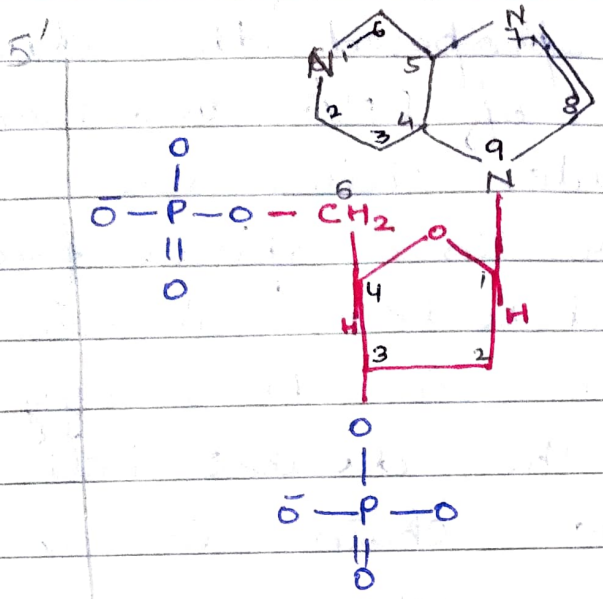

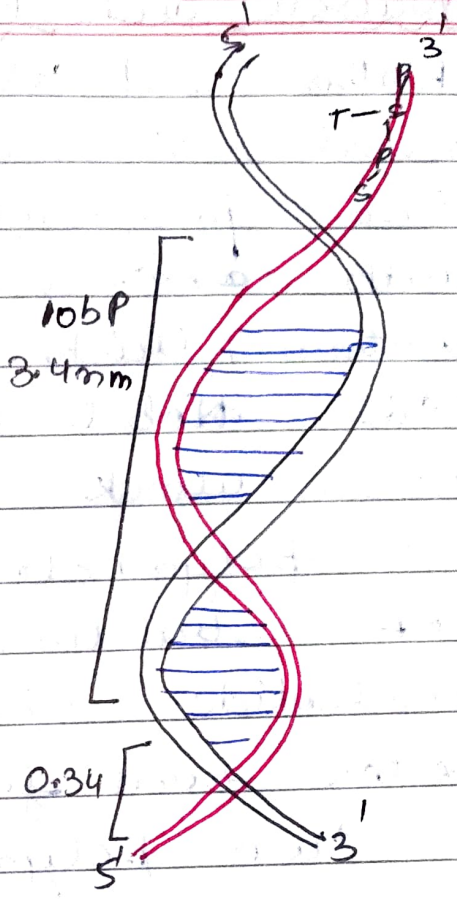

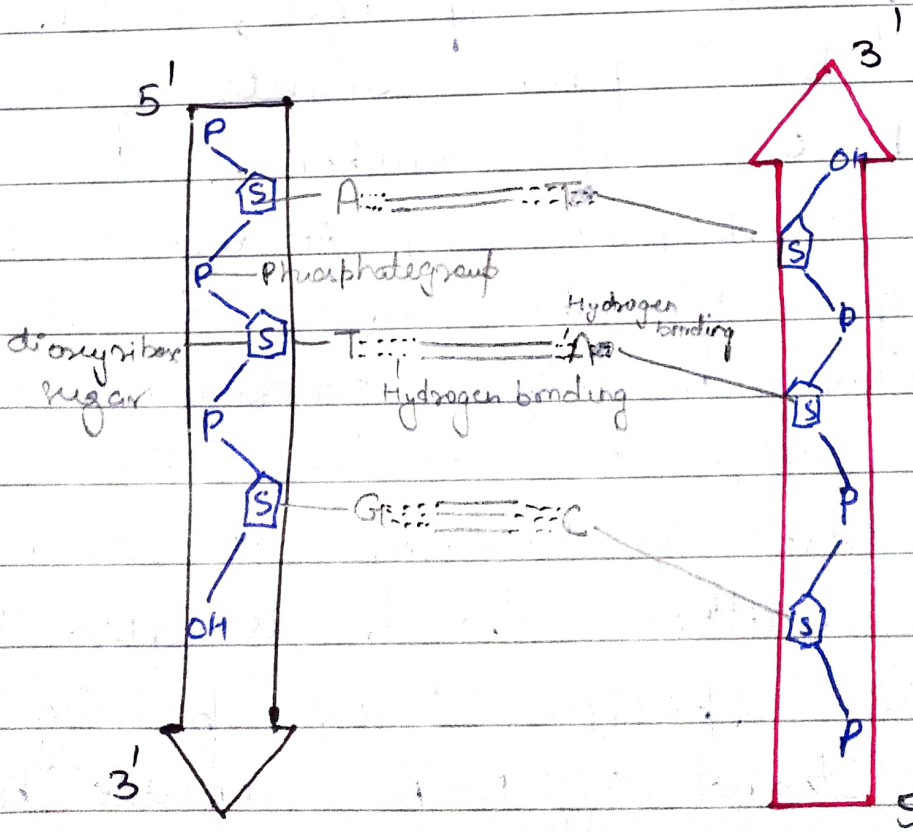

DNA: Structure and Replication.

DNA is the genetic material in all eleganisms. The genetic material is that a substance that occurs in every cell of an ouganism, stores biological information in coded form, and transmit it to the next generation, able to occassionally develop inheritable changes and causes the expression of information in the progency.

Structure:-

Chemical composition:- Chemical structure of DNA was explained by P.A. Levene. DNA molecule is a long double chain of deoxyribonucleotide. The two deoxyribonucleotide chains are unbranched and are twisted around a common axis to form a right-handed double helise that encloses a cylindrical space inside it. DNA molecule is a macro molecule compound of repeating subunits called nucleotides and each nucleotides consists of three ~~pentose~~ group ~~pen~~ phosphate group, pentose sugar and nitrogenous base.

1) **Bases** - DNA molecules contain two types of bases i) Purines
ii) Pyrimidines

Purines:- The nitrogenous base may be a 9 membered double-ringed ~~structure~~ purine.

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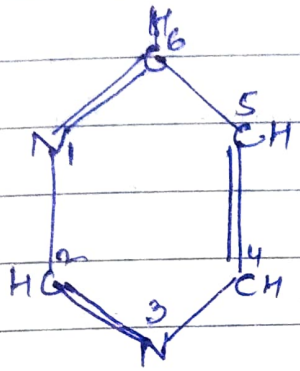
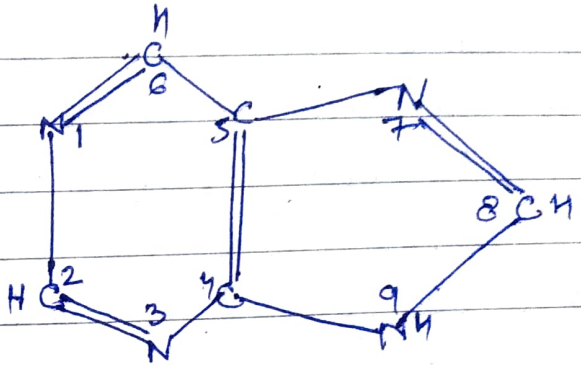
The DNA molecule contain two main types of purines – Adenine (A) and Guanine (G).
Pyrimidines – The nitrogenous base may be a 6-membered ~~ring~~ single ring - pyrimidine.
The DNA molecules contain two main types of pyrimidines – Thymine (T) and Cytosine (C).



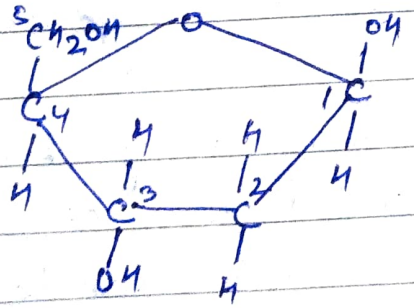
Pyrimidine



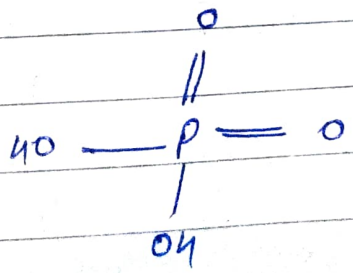
Purine

2) Sugar :- DNA molecules contain deoxyribose sugar.

3) Phosphoric Acid :- One molecule of phosphoric acid is linked with pentose sugar in each nucleotide at Carbon – 5.



Deoxyribose Sugar.



Phosphate.

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Double Helical Structure

The one of the most exciting breakthrough in the history of molecular biology occured in 1953. when James Watson and Francis crick discovered the correct structure of DNA molecule. Their double helix model suggest the mechanism for the transmission of genetic characters / information. The double helical structure was based on two major evidences:-

c) Chargaff's rule:- In 1950, Erwin Chargaff formulated important generalizations about DNA structure. These generalizations are called Chargaff's rule in his honour. They are summarized below:-

1) The purines and pyrimidines are always equal in amount i.e. A+G = T+C.
2) The amount of adenine is always equal to that of thymine & the amount of Guanine is always equal to Cytosine i.e. A = T and G = C
3) The base ratio $\frac{A+T}{G+C}$ may vary from one species to another, but is constant for a given species. This ratio can be used to identify the source of DNA, and can help in classification.
4) The deoxyribose sugar and phosphate components occur in equal proportions.

2) X-ray diffraction pattern :- Wilkins, Franklin and their co workers detect the x-ray diffraction of DNA molecule and they indicate that DNA was highly ordered two strand structure with repeating subunits along the axis of molecule.

On the basis of these two evidences Watson & Crick proposed that DNA is the right handed double helical structure in which two polynucleotide chains are coiled with one-another

Watson and Crick proposed the first satisfactory three dimensional structure of DNA in 1953. Watson, Crick and Wilkins shared the 1962 Nobel Prize in Physiology for their work on the double helical model.

The model suggests that-

1) DNA molecule consists of two polynucleotide chains which are twisted around each other in double helical fashion to form a double helix.

2) The backbone of DNA strand is made up of alternate deoxyribose and phosphate group to which the organic bases are attached.

3) The two strands of DNA molecule are coiled right handedly.

4) The two polynucleotide chains are held by hydrogen bonds b/w purine and pyrimidi of opposite strands. Pairing of bases is specific. i.e. A bond T and G bond C.

5) There are three hydrogen bonds b/w C and G and two hydrogen bond b/w A and T.

6) The two strands of a DNA molecule are complementary to each other and distance b/w them is 20A°.

7) Once the sequence of base is known, the sequence of base in another strand is also known because of specific base pairing.

8) The double helical linear polymer can be compared with a spiral stair-case. The distance b/w two successive steps is 0.34 A° and the angle b/w them is 36° so it takes 10 steps to have a complete turn. Thus, there are 10 base pairs b/w two turns of the helix.

b/w them is uniformly 20A.

Significance - 1) It acts as a carrier of genetic information from generation to generation
It controls directly or indirectly metabolic activities of cell.
It guides the protein synthesis inside the cell.

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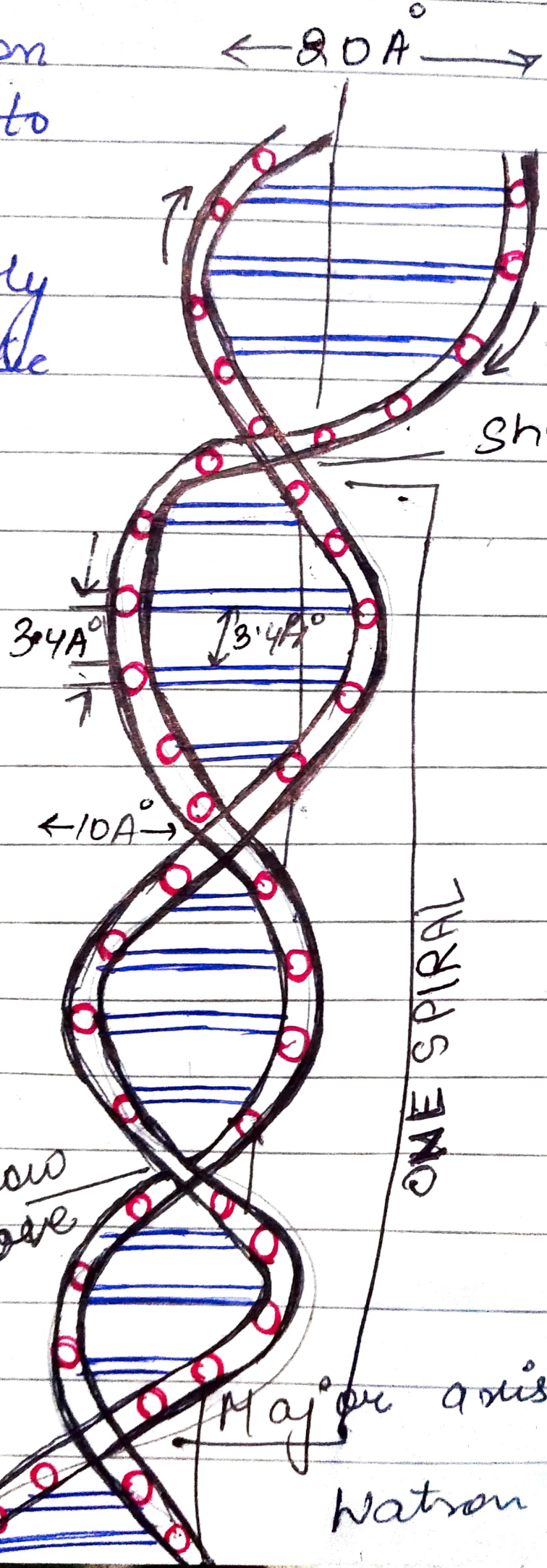


Watson & Crick model.

Watson and Crick Model

Types:-

1) Linear DNA :- Linear DNA has free ends. It is found in the nuclei of eukaryotic cells. It is associated with proteins. It is organised into a no. of chromosome, each containing one long DNA double helix.

2) Circular DNA :- Circulate DNA has its ends joined together. It is found in prokaryotic cells, and in the mitochondria and plastids of eukaryotic cells. It is not associated with protein. The prokaryotic DNA exists as a single chromosome.

A-DNA - The double helical structure of DNA described by Watson and Crick having right handed helical coiling. It contains 10 base pairs per turn of helix.

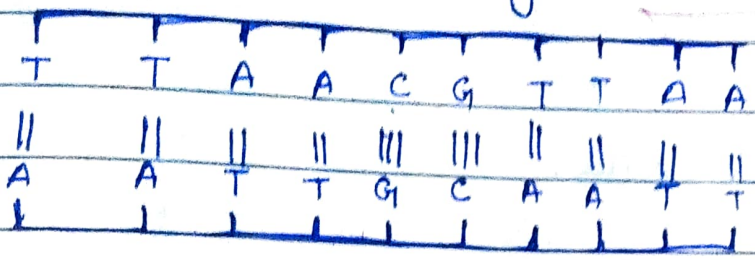
B-DNA - The double helical structure of DNA described by Watson and Crick having right handed helical coiling. It occurs naturally in living organisms.

C-DNA and D-DNA - They show right-handed double helical structure similar to B-DNA with minor differences. C-DNA has 9 base pairs per turn of the helix whereas D-DNA has only 8 base pairs.

Z-DNA - The double helical DNA molecule having left handed helical coiling is called Z-DNA. It was discovered by Alexander Rich in 1979. This form of DNA has been artifically produced in the laboratory. It has also been reported in the salivary gland / polytene chromosome of Drosophila. This type of DNA is biological significant and functions in the regulation of gene transcription.

Palindromic DNA - Meaning of palindrome is reading the same forward and backward. Some DNA sequences are palindromic. It has been observed that DNA molecules of several eukaryotes have palindromic sequences, in which the nucleotide sequence of one strand going in one direction is the same as the nucleotide sequence of the other strand going in the

other direction. Such DNA molecules having palindromic sequences were termed as palindromic DNA by Wilson and Thomas (1974).



Palindromic sequences.

Satellite or Repetitive DNA:- A DNA molecule consists of short identical DNA segments about 300 nucleotide, repeated several times and can be separated after DNA fragmentation and density gradient centrifugation as satellite band, is called satellite DNA (repetitive DNA). The prokaryotic DNA does not bear repetitive DNA sequences. These sequence of genome can replicate but fail to transcribe. Repetitive sequence DNA in higher organism with unknown function is called selfish DNA. In eukaryotes, there are two kinds of repeated sequences which can more about the genome. These are: Transposons and Retroposons.

Replication of DNA:- The process by which the parent DNA molecule give rise to two exactely similar sister DNA molecules, is called replication of DNA. The Watson-Crick model of DNA provides a replication mechanism.

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Types of DNA replication- There are three possible mechanisms by which the double stranded DNA molecule can replicate. These are-

1) Conservative.
2) Dispersive.
3) Semi- conservative.

1) Conservative Method- In this method, It implies that the original DNA molecule ~~can~~ replicate remains as such and a brand new copy of DNA molecule is synthesized from the old molecule.

2) Dispersive method- In this method, It implies that the parent DNA molecule undergoes disintegration and breaks up into its component nucleotides. These nucleotides along with newly synthesized nucleotides, synthesize two double stranded DNA molecules.

3) Semi- conservative Method- In this method, the two strands of DNA molecule separate from each other. Each strand then gets new nucleotides from the pool and synthesizes its complementary strand. Thus, one strand of each daughter DNA molecule is derived from the parent molecule and the other strand is formed anew.

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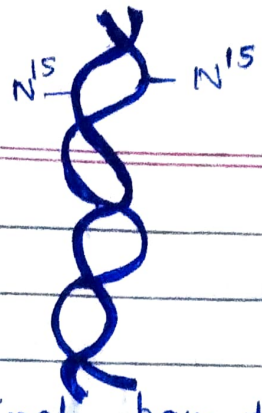
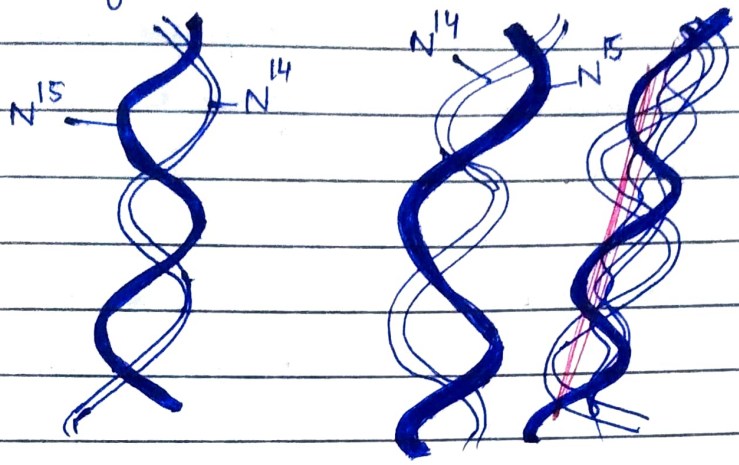
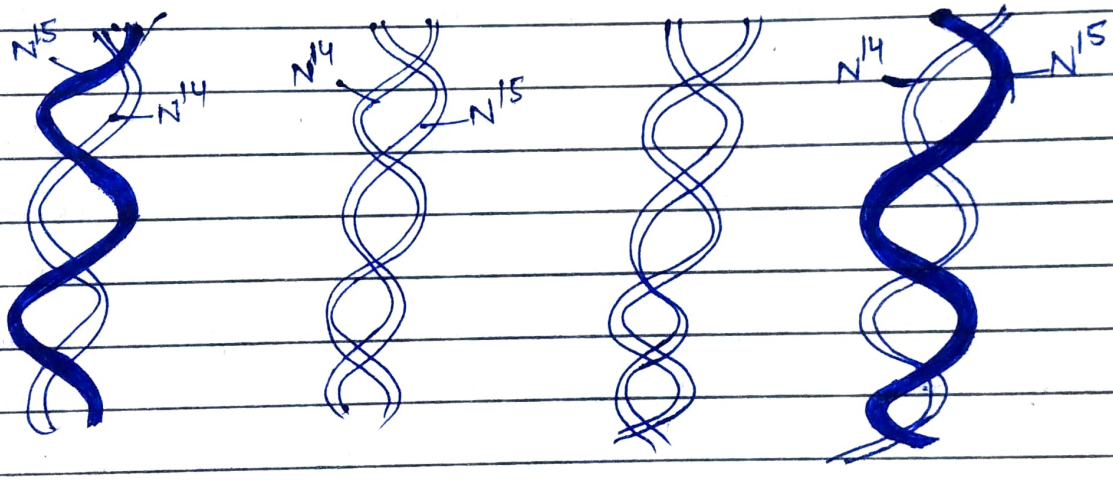


Original parent molecule.



First Generation Daughter molecules.



Second Generation Daughter molecules.

Semi-Conservative replication of DNA.

Requirement for DNA replication- The replication of DNA requires no. of enzymes, proteins, primers, nucleotides, ions. Some of them are listed below:

1) **Nucleotide** - Replication of DNA requires four kinds of nucleotides. Deoxyadenosine triphosphate

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(d ATP), Deoxyguanosine triphosphate (d GTP), Deoxycytidine triphosphate (dCTP) and Deoxythymidine triphosphate (dTTP). These nucleotides are formed from their monophosphates by ~~nucleoplasm~~ the process of phosphorylation catalysed by enzymes phosphorylase.

2) Enzymes - Replication of DNA requires presence of several enzymes which perform specific function. Some of them are -

a) Topoisomerases - These are concerned with breaking and resealing of DNA strand to relieve the tension of coiling.

b) Helicases - These enzymes unwind the helix of DNA

c) DNA polymerase III - This enzymes is responsible for DNA replication in 5' → 3' direction.

d) DNA polymerase I - It is a repair enzyme and fills in gaps b/w the fragments of DNA, synthesized by DNA polymerase.

e) RNA primase - This enzyme is responsible for synthesis of short segment of RNA, called RNA primer.

f) DNA ligase - This enzyme help in joining the DNA fragments.

~~3) Proteins~~ -

g) SSB - (Single Strand Binding protein) These enzyme help in uncoiling the structure of DNA. It prevent from rebinding

3) RNA primers - Replication of DNA in prokaryotes as well as eukaryotes is not possible until a short segment of RNA, called RNA primer is synthesized and initiates the process.

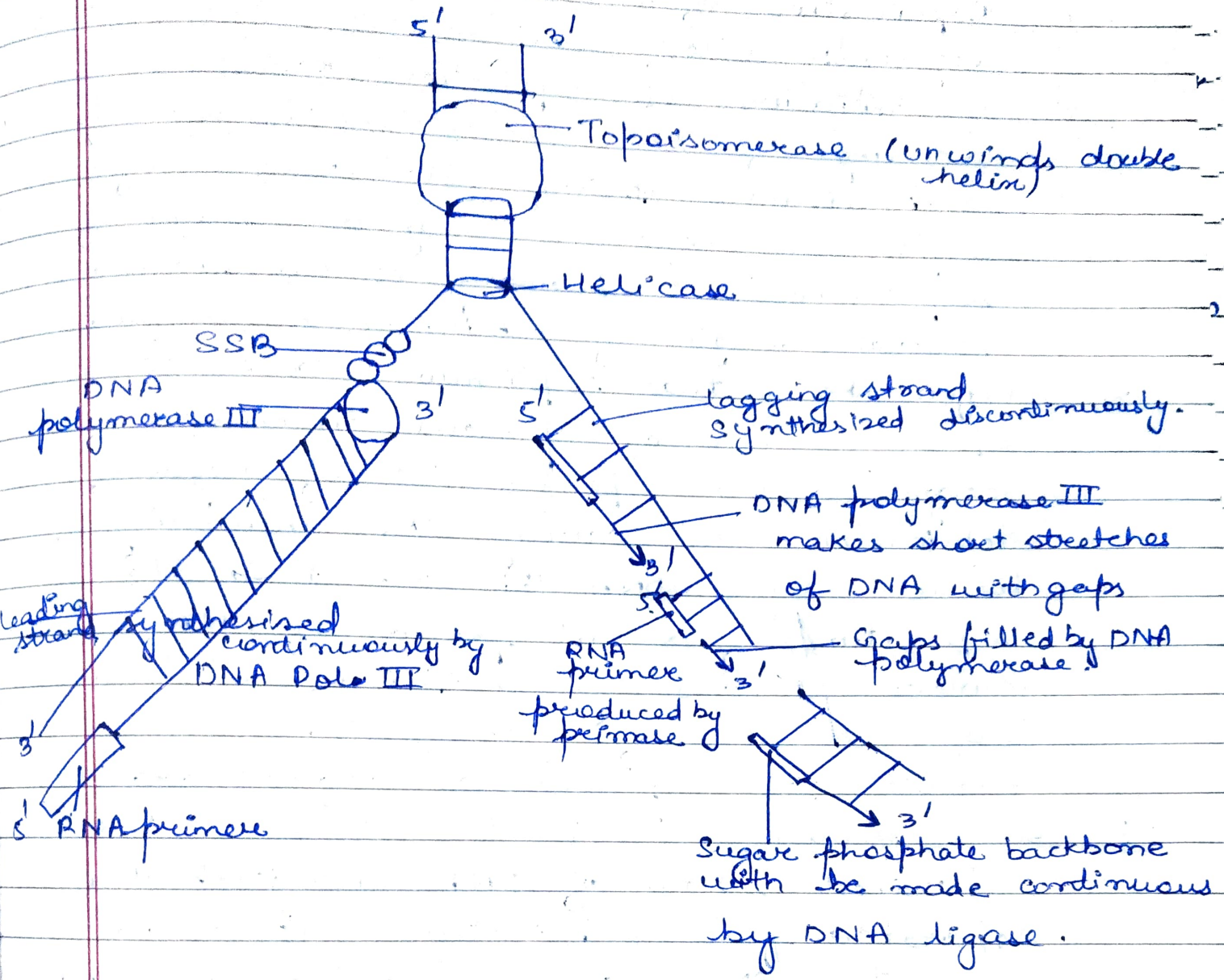

Fig Showing continuous replication of a daughter DNA steand on leading strand and discontinuous replication of lagging strand.

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Mechanism of Replication of DNA

Replication of DNA in prokaryotes and eukaryotes is semi-conservative type. A generalized mode of replications is given below :-

1) Replication of DNA occurs in a series of segments called replicons.
2) The initiation of replication begins i.e. uncoiling of the double helix begins from a particular point called origin of replication. There is only one origin of replication in prokaryotes and there are several origin of replication in eukaryotes.
3) Before the actual replication starts, some of the initiation proteins (unwindases) recognise the specific site for origin of replication and causing the unwinding of double helical DNA. This results in the separation of small portion of DNA molecule forming a kind of bubble or loop. In case of eukaryotes, several bubbles are formed at definite intervals.
4) Some protein molecules (SSB) bind the separated strand of DNA and keep them separated for replication. The two separated DNA strand function as templates.
5) Unwinding of DNA is followed by the synthesis of RNA primer on template strand. The primer is synthesized at the site of DNA replication with the help of enzyme RNA primase.

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6) Following the synthesis of RNA primer, the enzyme DNA polymerase III activated. This enzyme become active only in the presence of ~~DNA pol III~~ RNA primase. The enzyme DNA pol. III adds complementary deoxyribonucleotides (dATP, dGTP, dCTP, dTTP) to elongate the RNA primer. At 3'-OH terminal, the complementary deoxyribonucleotides are added to RNA primer by the formation of phosphodiester linkage liberating the pyrophosphates. Furthermore, the complementary deoxyribonucleotide get attached to the free bases of DNA template and a new strand is synthesized in 5' ⟶ 3' direction by the action of enzyme DNA pol. III.

7) Since the two strands of DNA runs in antiparallel direction and DNA pol. III replicates the DNA strand only in 5' ⟶ 3' direction. The replication process occurs on one strand in one direction and on another strand in opposite direction. On one strand, the synthesis of new DNA strand goes on continuously in 5' ⟶ 3' direction and this new strand is called leading strand. On the other strand, synthesis of new DNA strand is possible only in pieces. These pieces synthesized in 5' ⟶ 3' are called Okazaki fragments and the resultant strand is known as lagging strand. Thus, the replication of DNA is continuous semiconservative in one strand and discontinuous and semi-conservative in another strand.

8) DNA primer is ~~responsible~~ required for the

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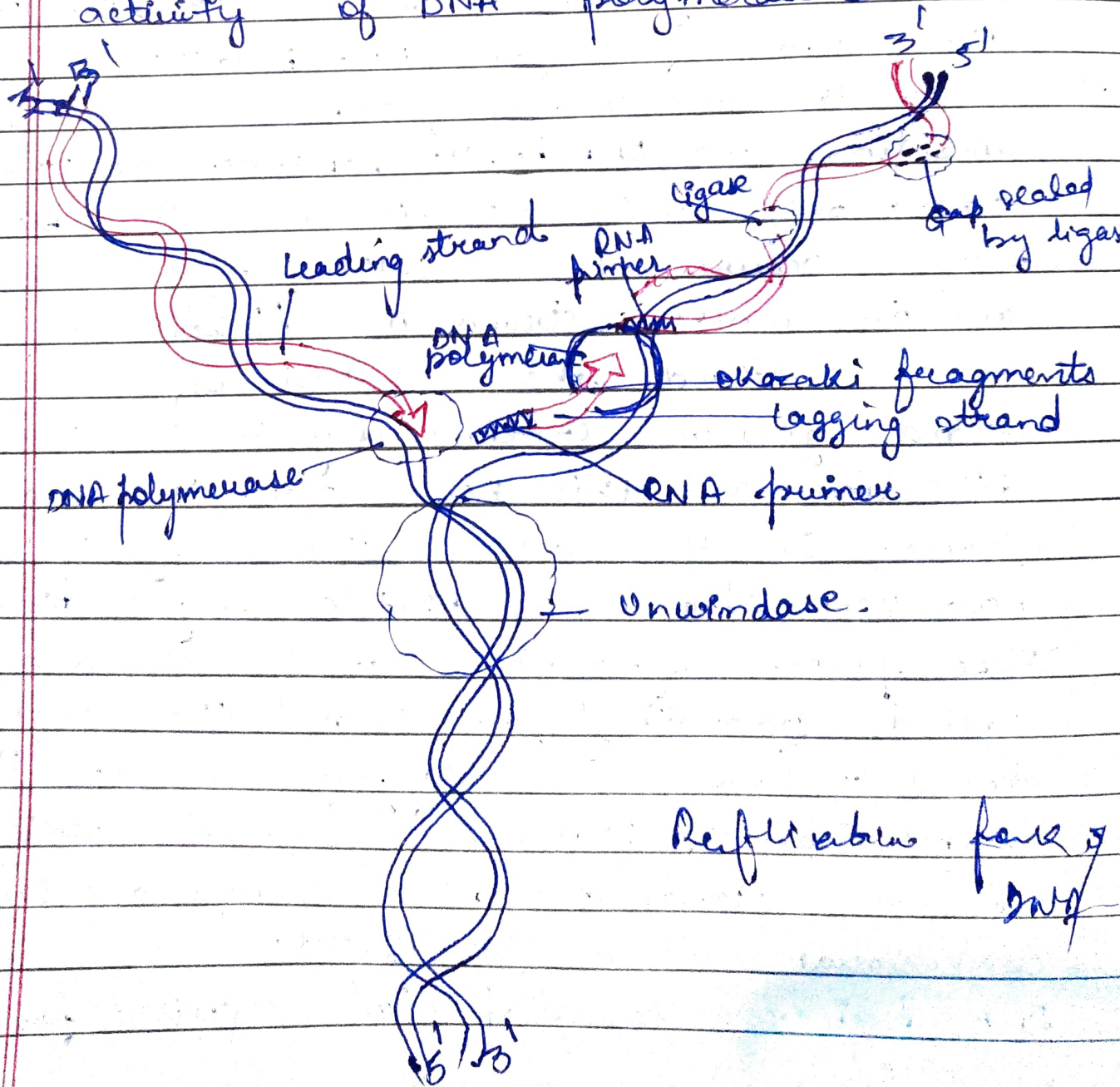
formation of Okazaki fragments. Okazaki fragments are joined togethere by DNA ligase.

Proof reading and DNA repair — They comprise the removal of mismatch nucleotides during replication and insertion of new correct complementary nucleotides in their places. Sometime DNA segments get damaged. In DNA repair system, these damaged portions are replaced with normal DNA segments. The proof reading and DNA repair is done by the activity of DNA polymerase I.



Plasmid → Plasmids are small double stranded DNA molecules that occure naturally in bacteria but outside the bacterial chromosome. They are considered as extra chromosomal material. A bacterial cell consists of one or more copies of plasmids. They are inherited from parent bacterial cell to daughter cell and they have capability to self replication in bacterial cytoplasm. The double stranded or circular DNA molecule of plasmid can be broken with the help of enzyme known as restriction nuclease or restriction enzyme or molecular scissor to yield a linear molecule which passes from one bacterial cell to another through the process of transformation. There are many of plasmid vectors and they vary from one another in size and comparition of genes. The plasmid consists of origin site and it is known as origin of replication under natural condition, the plasmid replicate to produce 20-30 copies per cell but in artificial medium in the presence of antibiotics the no. of can be increased upto thousand copies. The important feature of plasmid is to the presence of restriction site where restriction enzyme cut on these specific site so that a gene of interest or foreigner gene can be

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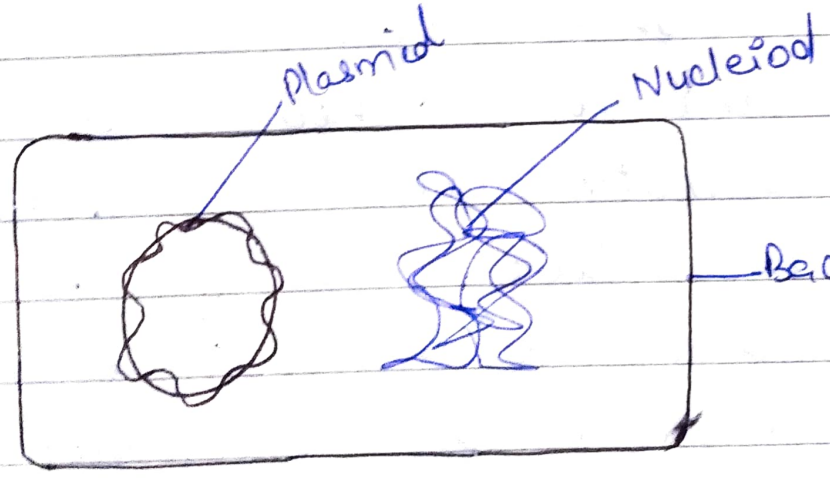
introduced into the plasmid cell - This process help in transmission of character from one cell to other cell of one organism to other organism) - Due to this unique feature plasmid is also called as cloning Vector.

Kinds of Plasmids

F- Plasmids
R- Plasmids
Cryptic Plasmids etc.



Oxidative phosphorylation and Electron Transport Chain

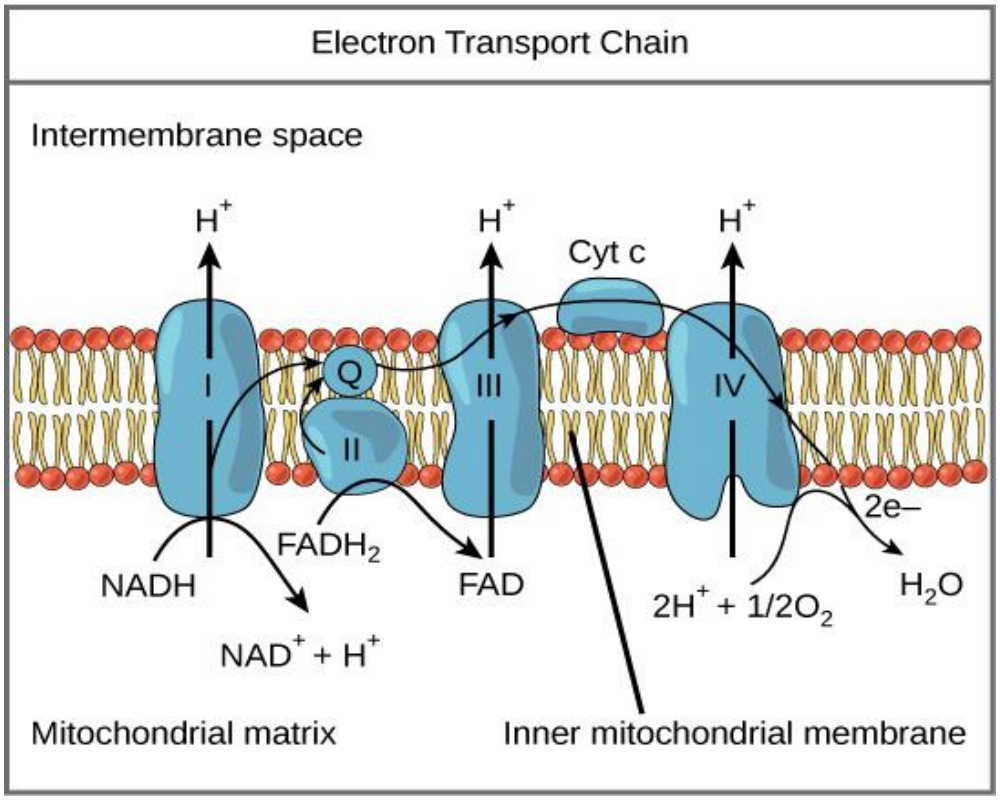
- Oxidative phosphorylation is the metabolic pathway in which electrons are transferred from electron donors to electron acceptors in redox reactions; this series of reactions releases energy which is used to form ATP.
- There are four protein complexes (labelled complex I-IV) in the electron transport chain, which are involved in moving electrons from NADH and $FADH_2$ to molecular oxygen.
- Complex I establish the hydrogen ion gradient by pumping four hydrogen ions across the membrane from the matrix into the intermembrane space.
- Complex II receives $FADH_2$, which bypasses complex I, and delivers electrons directly to the electron transport chain.
- Ubiquinone (Q) accepts the electrons from both complex I and complex II and delivers them to complex III.
- Complex III pumps protons through the membrane and passes its electrons to cytochrome c for transport to the fourth complex of proteins and enzymes.
- Complex IV reduces oxygen; the reduced oxygen then picks up two hydrogen ions from the surrounding medium to make water.

Oxidative phosphorylation is a highly efficient method of producing large amounts of ATP, the basic unit of energy for metabolic processes. During this process electrons are exchanged between a molecule, which creates a chemical gradient that allows for the production of ATP. The most vital part of this process is the electron transport chain, which produces more ATP than any other part of cellular respiration.

Electron Transport Chain

The electron transport chain is the final component of aerobic respiration and is the only part of glucose metabolism that uses atmospheric oxygen. Electron transport is a series of redox reactions that resemble a relay race. Electrons are passed rapidly from one component to the next to the endpoint of the chain, where the electrons reduce molecular oxygen, producing water. This requirement for oxygen in the final stages of the chain can be seen in the overall equation for cellular respiration, which requires both glucose and oxygen.

A complex is a structure consisting of a central atom, molecule, or protein weakly connected to surrounding atoms, molecules, or proteins. The electron transport chain is an aggregation of four of these complexes (labelled I through IV), together with associated mobile electron carriers. The electron transport chain is present in multiple copies in the inner mitochondrial membrane of eukaryotes and the plasma membrane of prokaryotes.



The electron transport chain: The electron transport chain is a series of electron transporters embedded in the inner mitochondrial membrane that shuttles electrons from NADH and $FADH_2$ to molecular oxygen. In the process, protons are pumped from the mitochondrial matrix to the intermembrane space, and oxygen is reduced to form water.

Complex I

To start, two electrons are carried to the first complex aboard NADH. Complex I is composed of flavin mononucleotide (FMN) and an enzyme containing iron-sulphur (Fe-S). FMN, which is derived from vitamin B_2 (also called riboflavin), is one of several prosthetic groups or co-factors in the electron transport chain. A prosthetic group is a non-protein molecule required for the activity of a protein. Prosthetic groups can be organic or inorganic and are non-peptide molecules bound to a protein that facilitate its function.

Prosthetic groups include co-enzymes, which are the prosthetic groups of enzymes. The enzyme in complex I is NADH dehydrogenase, a very large protein containing 45 amino acid chains. Complex I can pump four hydrogen ions across the membrane from the matrix into the intermembrane space; it is in this way that the hydrogen ion gradient is established and maintained between the two compartments separated by the inner mitochondrial membrane.

Q and Complex II

Complex II directly receives $FADH_2$, which does not pass through complex I. The compound connecting the first and second complexes to the third is ubiquinone (Q). The Q molecule is lipid soluble and freely moves through the hydrophobic core of the membrane. Once it is reduced to QH_2, ubiquinone delivers its electrons to the next complex in the electron transport chain.

Q receives the electrons derived from NADH from complex I and the electrons derived from $FADH_2$ from complex II, including succinate dehydrogenase. This enzyme and $FADH_2$ form a small complex that delivers electrons directly to the electron transport chain, bypassing the first complex.

Since these electrons bypass, and thus do not energize, the proton pump in the first complex, fewer ATP molecules are made from the $FADH_2$ electrons. The number of ATP molecules ultimately obtained is directly proportional to the number of protons pumped across the inner mitochondrial membrane.

Complex III

The third complex is composed of cytochrome b, another Fe-S protein, Rieske center (2Fe-2S center), and cytochrome c proteins; this complex is also called cytochrome oxidoreductase. Cytochrome proteins have a prosthetic heme group.

The heme molecule is similar to the heme in hemoglobin, but it carries electrons, not oxygen. As a result, the iron ion at its core is reduced and oxidized as it passes the electrons, fluctuating between different oxidation states: Fe^{2+} (reduced) and Fe^{3+} (oxidized).

The heme molecules in the cytochromes have slightly different characteristics due to the effects of the different proteins binding them, which makes each complex. Complex III pumps protons through the membrane and passes its electrons to cytochrome c for transport to the fourth complex of proteins and enzymes. Cytochrome c is the acceptor of electrons from Q; however, whereas Q carries pairs of electrons, cytochrome c can accept only one at a time.

Complex IV

The fourth complex is composed of cytochrome proteins c, a, and a_3. This complex contains two heme groups (one in each of the cytochromes a and a_3) and three copper ions (a pair of Cu_A and one Cu_B in cytochrome a_3).

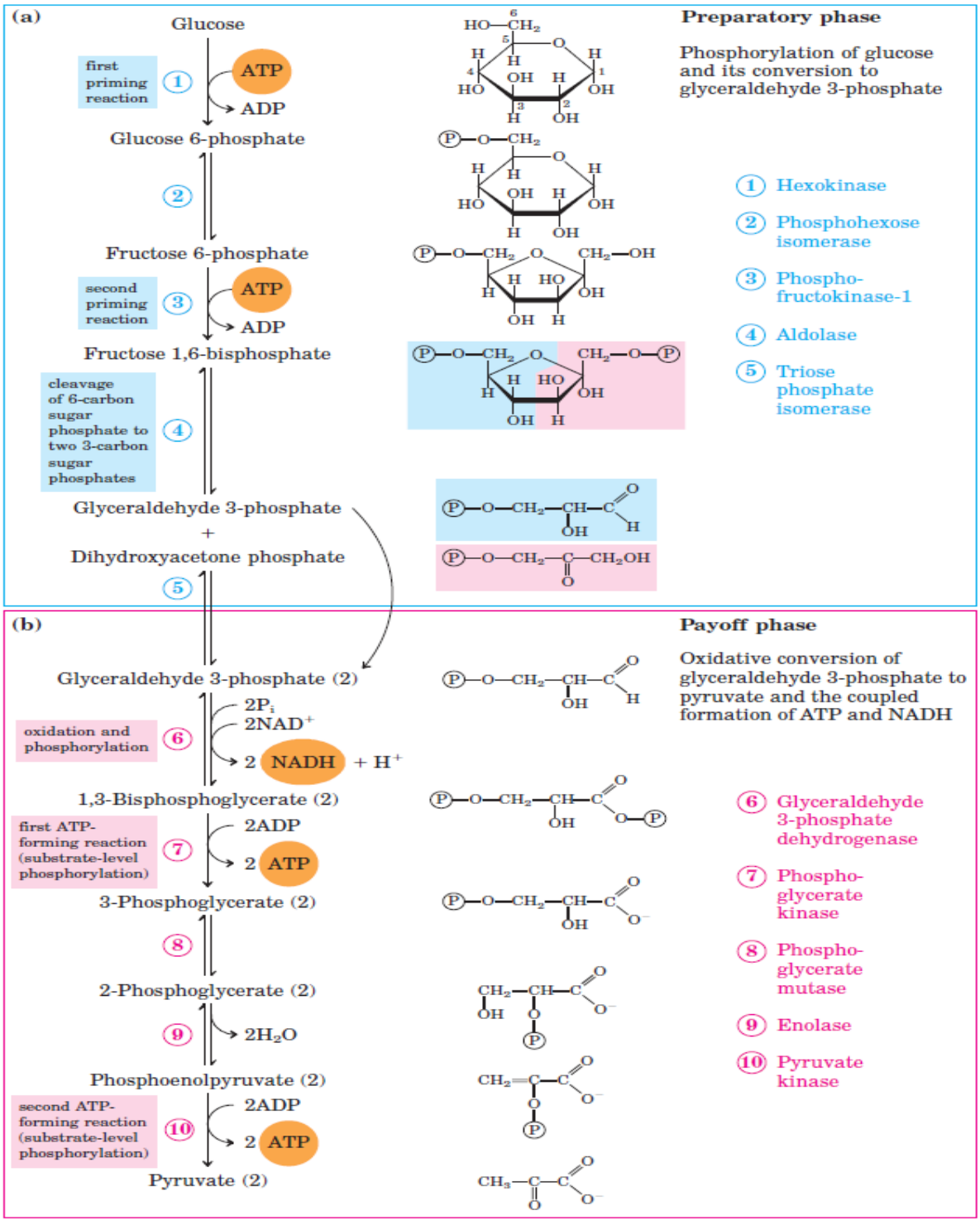
The cytochromes hold an oxygen molecule very tightly between the iron and copper ions until the oxygen is completely reduced. The reduced oxygen then picks up two hydrogen ions from the surrounding medium to produce water (H_2O). The removal of the hydrogen ions from the system also contributes to the ion gradient used in the process of chemiosmosis.

GLYCOLYSIS / EMBDEN – MEYER HOF – PARANAS (EMP) PATHWAY

Glycolysis can take place even in the absence of O_2.

One molecule of the **6** carbon compound, glucose is broken down through a series of enzyme reactions into two 3-carbon compounds, **the pyruvic acid.**

Glycolysis takes place in the **cytoplasm** and it does not require oxygen. Hence it is an anaerobic process.



Steps:

1. Glucose molecules react with ATP molecules in the presence of the enzyme hexokinase to form glucose -6- phosphate.

   Glucose + ATP → Glucose -6- phosphate + ADP

2. Glucose-6-phosphate is isomerised into fructose-6-phosphate in the presence of phospho hexose isomerase.

   Fructose + ATP → Fructose -6- phosphate + ADP

3. Fructose-6-phosphate reacts with one molecule of ATP in the presence of phospho hexo kinase forming fructose 1, 6-disphosphate.

   Fructose – 6- phosphate + ATP → Fructose -1,6- biphosphate + ADP

4. Fructose 1, 6 diphosphate is converted into two trioses,
   3-phospho glyceraldehyde and dihydroxy acetone phosphate in the presence of aldolase.

   Fructose -1,6- biphosphate → 3-phospho glyceraldehyde+ DHAP

5. 3-phosphoglyceraldehyde reacts with H_3PO_4 and forms 1,3-diphosphoglyceraldehyde where, the reaction is non –enzymatic.

6. 1, 3-Diphosphoglyceraldehyde is oxidized to form 1,3- diphosphoglycerate in the presence of triose-phosphate dehydrogenase and coenzyme NAD+.
   The NAD+ acts as hydrogen acceptor and reduced to NADH+ + H+ in the reaction.

   Glyceraldehde -3- phosphate + NAD + Pi → 1,3- diphosphoglycerate + NADH

7. 1, 3-Diphosphoglycerate reacts with ADP in the presence of phosphoglyceric transphorylase (kinase) to form 3 phosphoglyceric acid and ATP

   1,3- diphosphoglycerate + ADP → 3, Phosphoglycerate + ATP

8. 3, Phosphoglycerate → 2, Phosphoglycerate acid is isomerized into 2 phosphoglyceric acid in the presence of the enzyme, phospho glycero mutase.

3, Phosphoglycerate → 2, Phosphoglycerate

9. 2 phosphoglyceric acid is converted into 2-phosphoenolpyruvic acid in the presence of enolase.

2, Phosphoglycerate → Phosphoenol pyruvate + H2O

10. 2 phospho enol pyruvic acid reacts with ADP to form one molecule each of pyruvic acid and ATP in the presence of pyruvate kinase.

Phosphoenol pyruvate + ADP → Pyruvate + ATP .

Glycolysis or EMP pathway is common in both aerobic and anaerobic respiration

The overall glycolytic process can be summarized as follows

$C_6H_{12}O_6 + 2ATP + 2NAD + 4ADP + 2H_3PO_4$

$2\ CH_3COCOOH + 2ADP + 2NADH_2 + 4\ ATP$

Pyruvic acid

- Thus there is a gain of 4-2 = 2 ATP molecules per hexose sugar molecule oxidized during this process.
- Besides this, 2 molecules of reduced coenzyme NADH2 are also produced per molecule of hexose sugar in glycolysis.
- During aerobic respiration, these two $NADH_2$ are oxidized via the electron transport chain to yield 3 ATP molecules each. Thus **6** ATP molecules are formed.

INSERTION AND REPLACEMENT VECTORS

- Once the problems posed by packaging constraints and by the multiple restriction sites had been solved, the way was open for the development of different types of lambda-based cloning vectors.
- The first two classes of vector to be produced were **lambda insertion** and **lambda replacement** (or substitution) vectors.

INSERTION VECTORS {Lambda based}

- With an insertion vector (Figure 6.12a), a large segment of the non-essential region has been deleted, and the two arms ligated together.
- An insertion vector possesses at least one unique restriction site into which new DNA can be inserted.
- The size of the DNA fragment that an individual vector can carry depends, of course, on the extent to which the non-essential region has been deleted.
- **Two popular insertion vectors are:**

- **Lambda gt10** (Figure 6.12b), which can carry up to 8 kb of new DNA, inserted into a unique EcoRI site located in the cI gene.
- Insertional inactivation of this gene means that recombinants are distinguished as clear rather than turbid plaques.
- **Lambda ZAPII** (Figure 6.12c), with which insertion of up to 10 kb DNA into any of 6 restriction sites within a polylinker inactivates the lacZ' gene carried by the vector. Recombinants give clear rather than blue plaques on X-gal agar.

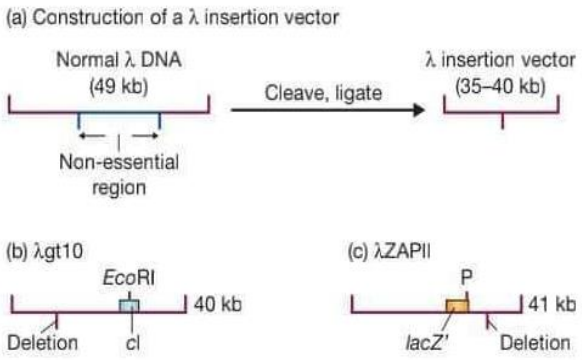


Figure 6.12
λ insertion vectors. P = polylinker in the *lacZ'* gene of λZAPII, containing unique restriction sites for *Sac*I, *Not*I, *Xba*I, *Spe*I, *Eco*RI, and *Xho*I.

To be continued in next lecture……

Krebs Cycle

Or

Tricarboxylic Acid (TCA) Cycle

Or

The citric Acid Cycle

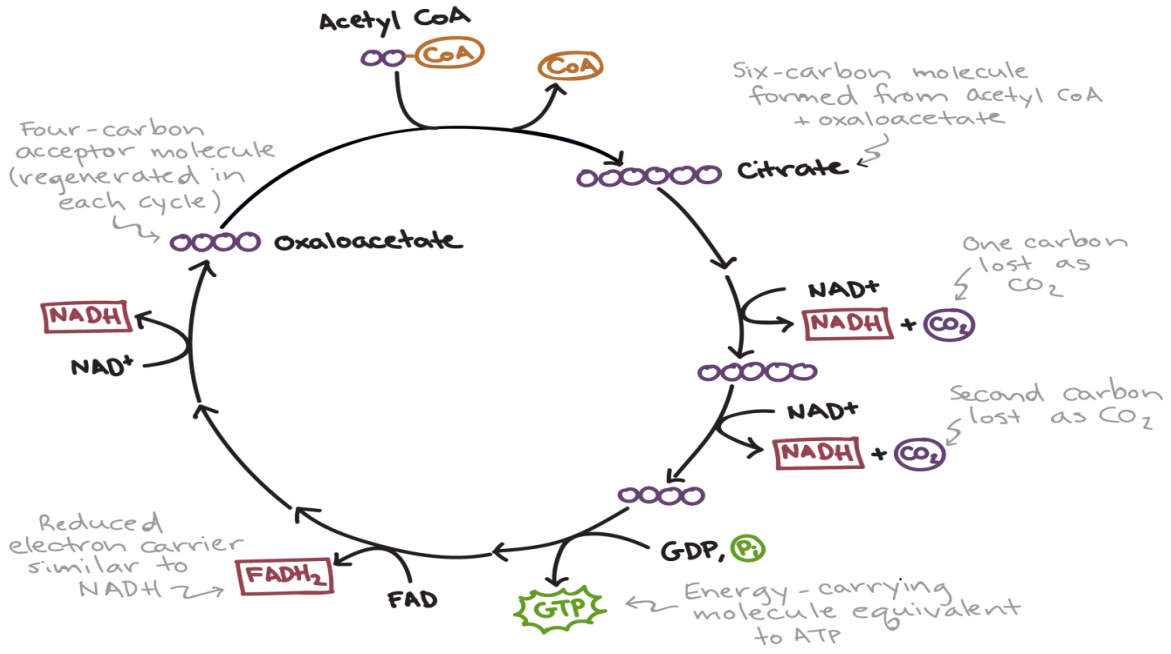
If the molecular oxygen is available, aerobic respiration takes place and the **pyruvate** produced in **glycolysis** in **cytosol** enters into **mitochondria** for further oxidation through Krebs cycle.

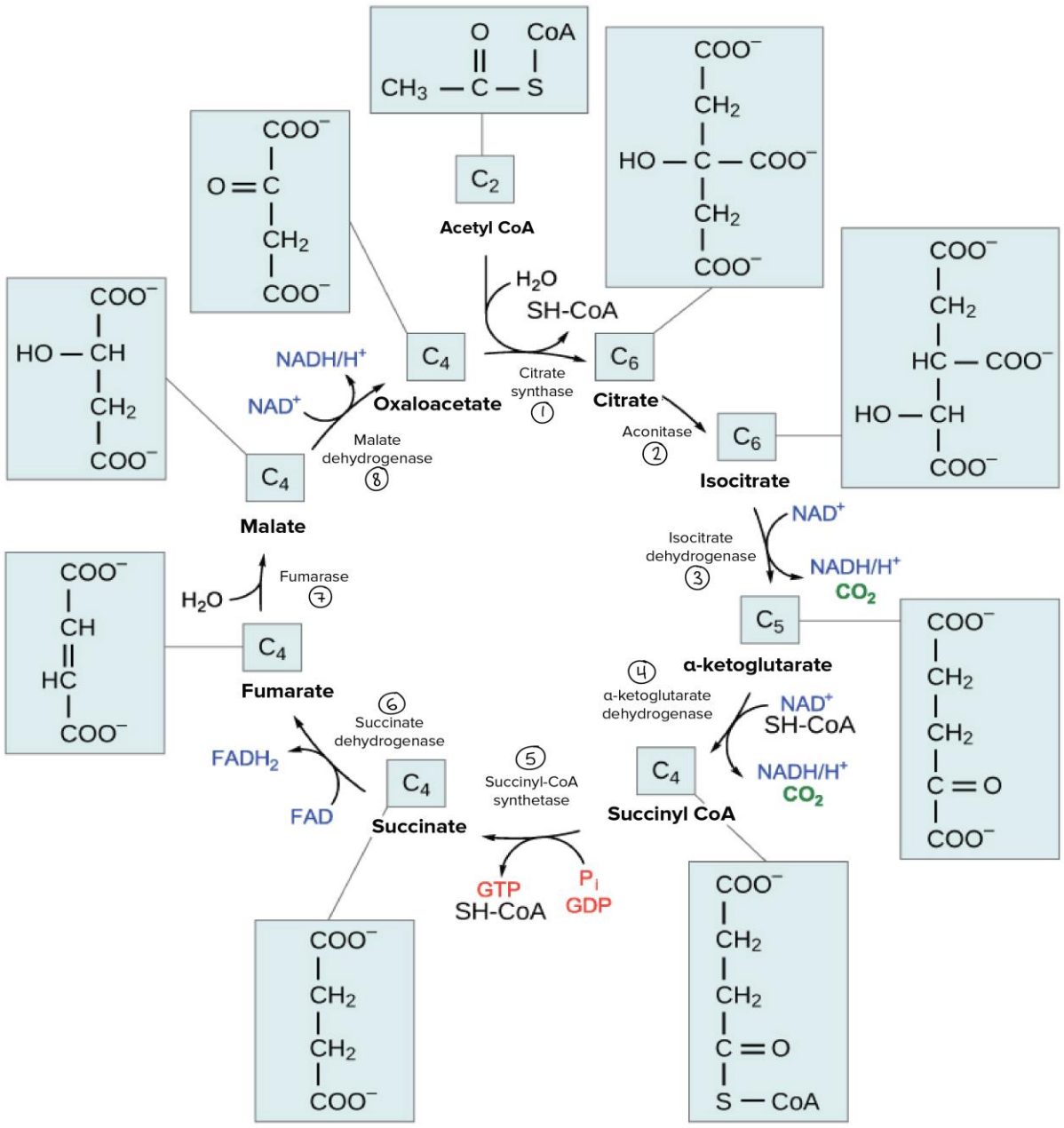
Krebs cycle is also known as Citric acid cycle or TCA because **citric acid** is an early intermediate of this cycle which contains **three carboxylic groups**.

Krebs cycle is so named after its discoverer **Hans A Krebs** who discovered this cycle in 1937 and was awarded the Nobel Prize in 1953 in Physiology category.

Overview of the citric acid cycle

In eukaryotes, the citric acid cycle takes place in the matrix of the mitochondria, just like the conversion of **pyruvate** to **acetyl CoA**. In prokaryotes, these steps both take place in the cytoplasm. All enzymes necessary for TCA cycle are found in **matrix** region of mitochondria. However, the enzymes **Succinate dehydrogenase** is present on the inner mitochondrial membrane.





Krebs Cycle

Steps of the citric acid cycle or Krebs cycle

Step 1. In the first step of the citric acid cycle, **acetyl CoA** joins with a four-carbon molecule--- **oxaloacetate**, releasing the CoA group and forming a six-carbon molecule called **citrate.**

Step 2. In the second step, **citrate** is converted into its isomer, **iso-citrate.** This is actually a two-step process, involving first the removal and then the addition of a water molecule, which is why the citric acid cycle is sometimes described as having nine steps—rather than the eight listed here.

Step 3. In the third step, isocitrate is oxidized and releases a molecule of carbon dioxide, leaving behind a **five**-carbon molecule—**α-ketoglutarate.** During this step, **NAD^+** is reduced to form **NADH.** The enzyme catalyzing this step, **isocitrate dehydrogenase**, is important in regulating the speed of the citric acid cycle.

Step 4. The fourth step is similar to the third. In this case, it's α-ketoglutarate that's oxidized, reducing NAD^+ to NADH and releasing a molecule of carbon dioxide in the process. The remaining four-carbon molecule picks up **Coenzyme --A,** forming the unstable compound **succinyl CoA.** The enzyme catalyzing this step, **α-ketoglutarate dehydrogenase**, is also important in regulation of the citric acid cycle.

Step5. In step five, the CoA of **succinyl CoA** is replaced by a phosphate group, which is then transferred to ADP to make ATP. In some cells, GDP—guanosine diphosphate—is used instead of ADP, forming GTP—guanosine triphosphate—as a product. The four-carbon molecule produced in this step is called **succinate.**

Step 6. In step six, succinate is oxidized, forming another four-carbon molecule called **fumarate.** In this reaction, two hydrogen atoms—with their electrons—are transferred to FAD, producing $FADH_2$. The enzyme that carries out this step is embedded in the inner membrane of the mitochondrion, so $FADH_2$ can transfer its electrons directly into the electron transport chain.

Step 7. In step seven, water is added to the four-carbon molecule fumarate, converting it into another four-carbon molecule called **malate.**

Step 8. In the last step of the citric acid cycle, **oxaloacetate**—the starting four-carbon compound—is regenerated by **oxidation of malate.** Another molecule of NAD^+ is reduced to NADH in the process.

Products of the citric acid cycle

Tracing the fate of the carbons that enter the citric acid cycle and counting the reduced electron carriers—NADH and $FADH_2$ and ATP produced.

In a single turn of the cycle,

- **Two carbons** enter from acetyl CoA and **two molecules** of carbon dioxide are released;
- **Three** molecules of NADH and one molecule of $FADH_2$ are generated; and
- **One** molecule of ATP or GTP is produced.

These figures are for one turn of the cycle, corresponding to one molecule of acetyl CoA. **Each glucose** produces **two acetyl CoA** molecules, so we need to multiply these numbers by 2 if we want the per-glucose yield.

Two carbons—from acetyl CoA—enter the citric acid cycle in each turn, and two carbon dioxide molecules are released. However, the carbon dioxide molecules don't actually contain carbon atoms from the acetyl CoA that just entered the cycle.

Instead, the carbons from **acetyl CoA** are initially incorporated into the intermediates of the cycle and are released as carbon dioxide only during later turns. After enough turns, all the carbon atoms from the acetyl group of acetyl CoA will be released as **carbon dioxide.**

Where's all the ATP?

Output of the citric acid cycle seems pretty unimpressive. All that work for just one ATP or GTP?

It's true that the citric acid cycle doesn't produce much ATP directly. However, it can make a lot of ATP indirectly, by way of the NADH and $FADH_2$ it generates. These electron carriers will connect with the last portion of cellular respiration, depositing their electrons into the electron transport chain to drive synthesis of ATP molecules through **oxidative phosphorylation.**

Maize: Origin, Botany and Cultivation

Maize or corn (Zea mays) is a plant belonging to the family of grasses (Poaceae). It is cultivated globally being one of the most important cereal crops. Maize is a versatile crop grown over a range of agro-climatic zones. Maize is the third most important food grain in India after wheat and rice. Maize is the third most important food grain in India after wheat and rice.

Origin

The center of origin for Z. mays has been established as the Mesoamerican region, now Mexico and Central America. Archaeological records suggest that domestication of maize began atleast 6000 years ago, occurring independently in regions of the south-western United States, Mexico, and Central America.

The Portuguese introduced maize to Southeast-Asia from the America in the 16th century. The maize was introduced into Spain after the return of Columbus from America and from Spain it went to France, Italy and Turkey.

In India, Portuguese introduced maize during the seventeenth century. From India, it went to China and later it was introduced in Philippines and the East Indies. Corn now is being grown in USA, China, Brazil, Argentina, Mexico, South Africa, Rumania, Yugoslavia and India.

Morphology

Maize is a tall, determinate, monoecious, annual C_4 plant varying in height from 1 to 4 metres producing large, narrow, opposite leaves (about a tenth as wide as they are long), borne alternately along the length of a solid stem.

The botanical features of various plant parts are as follows:

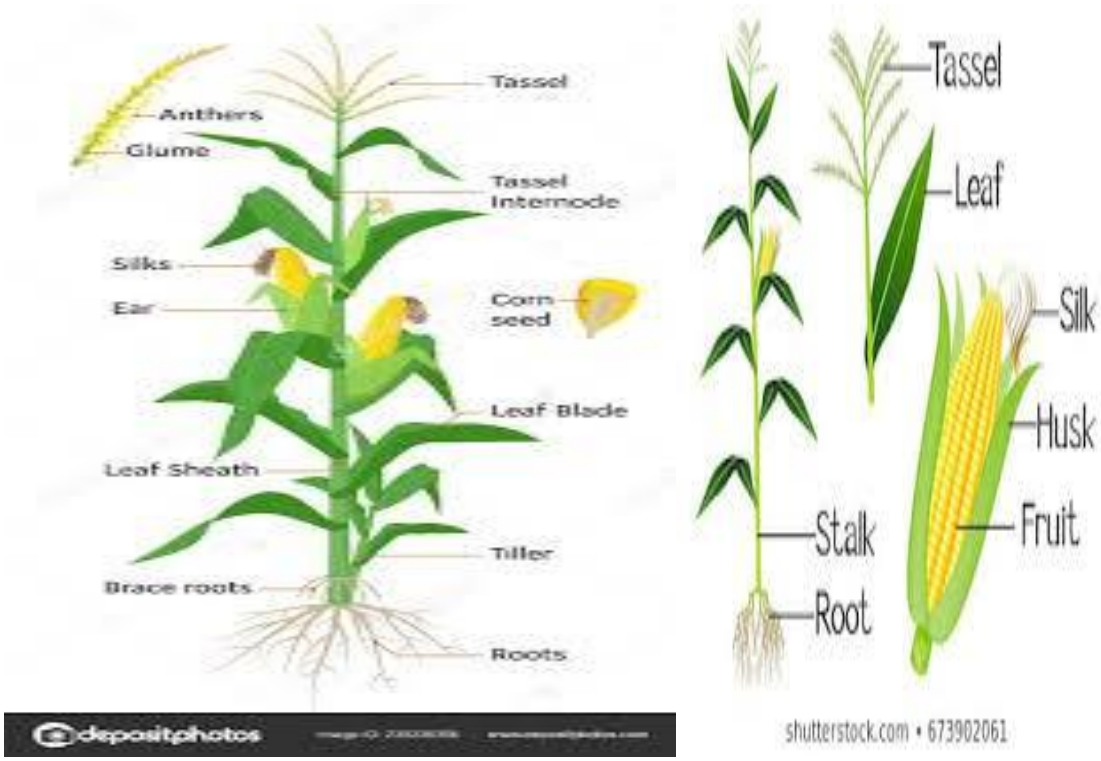
Root: Normally maize plants have three types of roots,

i) seminal roots - which develop from radicle and persist for long period

ii) ii) Adventitious roots - fibrous roots developing from the lower nodes of stem below ground level which are the effective and active roots of plant.

iii) Brace or prop roots - produced by lower two nodes. The roots grow very rapidly and almost equally outwards and downwards. Favourable soils may allow corn root growth up to 60 cm laterally and in depth.

Stem: The stem generally attains a thickness of three to four centimeters. The stem bears nodes and internodes. The upper leaves in corn are more responsible for light interception and are major contributors of photosynthate to grain.



Flower: The apex of the stem ends in the tassel, an inflorescence of male flowers and the female inflorescences (cobs or ears) are borne at the apex of condensed, lateral branches known as shanks protruding from leaf axils. The male (staminate) inflorescence, a loose panicle, produces pairs of free spikelets each enclosing a fertile and a sterile floret.

The female (pistillate) inflorescence, a spike, produces pairs of spikelets on the surface of a highly condensed rachis (central axis, or "cob"). The female flower is tightly covered over by several layers of leaves, and so closed in by them to the stem that they don't show themselves easily until emergence of the pale yellow silks from the leaf whorl at the end of the ear.

The silks are the elongated stigmas that look like tufts of hair initially and later turn green or purple in colour. Each of the female spikelets encloses two fertile florets, one of whose ovaries will mature into a maize kernel once sexually fertilized by wind-blown pollen.

Grain: The individual maize grain is botanically a **caryopsis**, a dry fruit containing a single seed fused to the inner tissues of the fruit case. The seed contains two sister structures, a germ which includes the plumule and radical from which a new plant will develop, and an endosperm which will provide nutrients for that germinating seedling until the seedling establishes sufficient leaf area to become autotrophy.

Cultivation of Maize

Climate: Maize crop is primarily a warm weather crop and it is grown in wide range of climatic conditions. In India, maize is traditionally grown in monsoon (Kharif) season, which is accompanied by high temperature (<35° C) and rains.

Soil: Soil texture is a foremost requirement as it controls moisture and nutrient capacity. Loam or silt loam surface soil and brown silt clay loam having fairly permeable sub soil are the ideal soil types for cultivation of maize.

Sowing Time: Kharif season crop: Seed is sowed in the month of june/july.

Land Preparation and Manuring: A well prepared flat-beds which has given 4-5 deep ploughing provided an ideal condition for sowing of crop.

1. FYM or compost: 5 tonne / ha

2. N: 100-120 Kg

3. P2 O5: 60 Kg

4. K2O/ 30-40 Kg

Method of Planting:

1. Seed treatment: Treated with Bavistan@ 3gm/Kg of seeds before sowing in the field.

2. Depth of Sowing: 4-6 cm, where maize is generally sown on flat beds.

3. Spacing: 70cm x 25cm for row to row and plant to plant.

4. A healthy seeds of 20-22Kg required for cultivation in one hectare of land.

Irrigation: For Kharif Maize, irrigation at early knee-high, tasselling and 50% silking

stages are to be given.

Variety: Hybrid variety – Ganga hybrid makka-5, Himalayan hybrid Makka.

The composite variety, Prabhat and Dhawal are specially recommended for cultivation in North-eastern hill regions.

Intercropping: Growing of one row of soybean in between 2 rows of maize (60 cm spacing) gave increase in yield of maize. The planting of one row of Maize alternating every 4 rows of Urdbean or black gram (30cm spacing) is found to be most suitable, resulted in the highest productivity.

Harvesting and Threshing: Maize is ready for harvesting even when the stacks and leaves are somewhat green but the husk cover has dried and turned brown. Conventional harvestor combines can be used for threshing Maize with husk to save labour involved in dehusking. The Maize ears should preferably be dried for 3-4 days after harvesting to improve grain

recoveries and reduce breakage losses during shelling.

Status of Maize Cultivation: Maize occupies an important place in Indian Agriculture. It is the third most important cereal in India after wheat and rice. The major maize growing states are Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh, Punjab, Andhra Pradesh, Himachal Pradesh, West Bengal, Karnataka and Jammu & Kashmir, jointly accounting for over 95% of the national maize production

Utilization: Maize is not only an important food crop for human consumption, but also a basic element of animal feed and raw material for manufacturing of many industrial products. The products include corn starch, maltodextrins, corn oil, corn syrup and products of fermentation and distilleries. It is also being recently used in the production of biofuel.

In India, about 28% of maize produced is used for food purpose, about 11% as livestock feed, 48% as poultry feed, 12% in wet milling industry (for example starch and oil production) and 1% as seed.

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Assistant Professor
GDC Hiranagar

Mechanism of Photosynthesis

Photosynthesis: It is the process by which green plants and certain bacteria such as blue green algae can make their own food in the presence of sunlight using water and CO_2 as raw material.

Photosynthesis is a complex process of synthesis of organic food materials. It is a complicated **oxidation- reduction** process where water is oxidized and CO_2 is reduced to carbohydrates.

The mechanism of photosynthesis consists of two parts.

1. Light reaction / Primary photochemical reaction / Hill's reaction/ Arnon's cycle

2. Dark reaction / Black man's reaction / Path of carbon in photosynthesis.

1. Light reaction or Primary photochemical reaction or Hill's reaction:

In Light reaction, **ATP and $NADPH_2$** are produced.

In the Dark reaction, **CO_2** is reduced with the help of ATP and $NADPH_2$ to produce **glucose**.

The light reaction is called **PRIMARY PHOTOCHEMICAL REACTION** ----as it is induced by light.

OR

Light reaction is also called as **Hill's reaction** as Hill proved that chloroplast produce O_2 from water in the presence of light.

OR

It is also called as **Arnon's cycle** because Arnon showed that the **H^+ ions** released by the break -down of water are used to reduce the coenzyme NADP to NADPH.

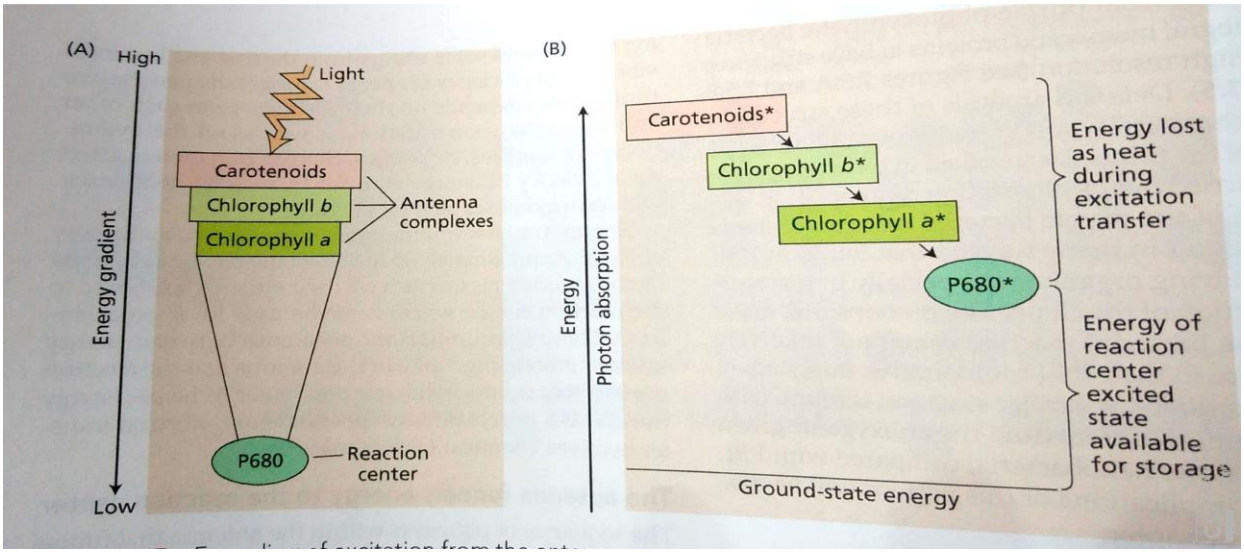
Process of light Reaction

Light reaction includes photophosphorylation as ATP is synthesized in the presence of light. The reaction takes place only in the presence of light in **Grana** portion of the **Chloroplast** and it is **faster** than dark reaction.

The chlorophyll absorbs the light energy and hence the chlorophyll is called **as photosystem or pigment system.** Chlorophylls are of different types and they absorb different wavelengths of light.

Chlorophylls exist in **two** photo systems---------- **Photosystem I (PSI)** and **Photosystem II (PS II).**

Both photo systems (PSII &PS I) are affected by light with wavelengths **shorter** than 680nm, while PS I is affected by light with wavelengths **longer** than 680nm.



The light reaction can be studied under the following headings.

A) Absorption of light energy by chloroplast pigments :

Different chloroplast pigments absorb light in different regions of the visible part of the spectrum.

B) Transfer of light energy from accessory pigments to chlorophyll a.

All the photosynthetic pigments except **chlorophyll a** are called as **accessory or antenna pigments.** The light energy absorbed by the accessory pigments is transferred by resonance to chlorophyll a which alone can take part in photochemical reaction. Chlorophyll a molecule can also absorb the light energy directly.

In pigment system **I**, the photoreaction centre is **P700** and in pigment system **II**-- it is **P680**.

C). Activation of chlorophyll molecule by photon of light.

When P700 or P680 forms of chlorophyll a receives a photon (quantum) of light, becomes an excited molecule having more energy than the ground state energy.

After passing through the unstable second singlet state and first singlet stage the chlorophyll molecules comes to the meta stable triplet state. This excited state of chlorophyll molecule takes part further in primary photochemical reaction.

D). Photolysis of water and O_2 evolution (oxidation of water).

These processes are associated with pigment system II and are catalyzed by Mn^{++} and Cl^- ions. When pigment system II is active ----it receives the light.

The water molecules split into OH^- and H^+ ions (Photolysis of water).

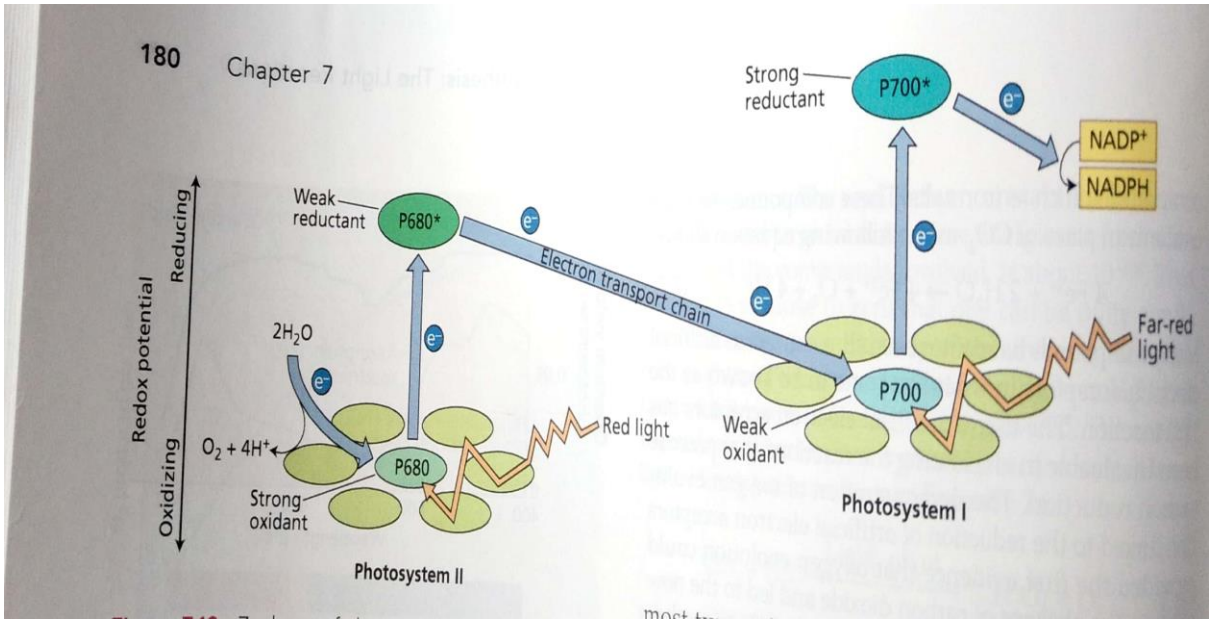
The OH^- ions unite to form some water molecules again

Release O_2 and electrons.

E). Electron transport and production of assimilatory powers ($NADPH_2$ and ATP).

It has already been observed that when chlorophyll molecule receives the photon of light, an electron is expelled from the chlorophyll a molecule along with extra energy. This electron after traveling through a number of electron carriers is utilized for the production of $NADPH_2$ from NADP and also utilized for the formation of ATP molecules from ADP and inorganic phosphate (Pi).

The transfer of electrons through a series of coenzymes is called **ELECTRON TRANSPORT** and the process of formation of ATP from ADP and Pi using the energy of electron transport is called as **PHOTOSYNTHETIC PHOSPHORYLATION OR PHOTOPHOSPHORYLATION**.



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MUTATION ⇒ Mutation is the sudden heritable changes in the genome (genetic material) of the cell or organisms. Mutation provides raw material for evolution. The term mutation was introduced by Hugo di Vries. Hereditary performed during mutation given by T.H. Morgan.

OR

Sources of variation in which new species are evolved. Or evolution of new species take place.

* Peter has autosomal recessive disorder called Xeroderma pigmentosum in which skin cells are sensitive to sunlight (UV). Causing cancer in human beings.

⇒ On the basis of origin, mutation are of two types :- 1) Natural mutation or ← Changes in Replication, cell division ← Spontaneous mutation.

2) Artifical mutation or ← Induced mutation. Temp. Increase

⇒ On the basis of size genome, mutation are of two types :- 1) Chromosomal mutation or ← Structural or No. changes ← macro mutation.

2) Gene or point mutation. ← Purine = Pyrimidine, Base nucleotide PC₁ ≡ C

⇒ On the basis of mutation sites : On the basis of cell tissues where mutation occurs, of two types : 1) Somatic mutation.

2) Germinal mutation.

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On the basis of mutation sites: on the basis of cell & tissues where mutation occur, two types:

1) Somatic mutation 2) Germinal mutation.

1) Somatic mutation → This type of mutation occurs in vegetation cells and known as somatic mutation. Spontaneous mutation which occur in somatic cells are called as sports.

2) Germinal mutation → This type of mutation occurs in germ cells and is most important for the transfer of character from parents to offsprings. This type of mutation occurs in heterozygous. In crop plants, germinal mutation are induced during sexual reproduction by cloning or vegetative propagation.

The agents which causes mutation known as mutagens.

Physical mutagens: The physical mutagens such as: X-rays
γ-rays
Neutrons
α-particles.
β-particles and they have high energy with ~~ionisation~~ ionizing radiation.
Non-ionizing radiation with low energy.

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Chemical mutation: 1) Ethyium bromide
2) Nitrous oxide
3) Colchichine
4) Mustard gas.

The induced mutation which is used in crop improvement

Role of mutation breeding in crop improvement

1) New varieties
2) New combinations
3) Vigour, taste, size
4) Qualitative traits
5) Quantative traits
6) Disease resistance
7) Stress resistance ⟶ ROS Reactive oxygen spp.
8) i Plant varieties
9) ii Drought
10) iii Saline
11) iv Temperature.

Tuesday.
-09-19

Transposable Genetic elements
OR
Transposons
OR
Jumping genes / moving elements.

I. History / Discovery

Maize crop: staple food crop / important main crop of the world.

I. Types of Transposons: Categories: Three:

1) Cut and paste Transposon } Enzyme - Transposase
2) Replicative Transposon
3) Retro transposon } RNA ⟶ DNA eg:- Revese transcriptase

Mobile genetic elements were first observed by Barbara Mc Clintock (1951) in maize plant while working with maize plants, she observed an uneven pigmentation pattern of the kernels.

when the relationship b/w the pigmentation pattern the spots were varying in size & colour and chromosome changes were examined. It was found that variegation in the colour of kernels was accompanied by transposition of genetic elements within or b/w the chromosome. It was then observed that genes are not always remain fixed but they occasionally move. The mobile genetic elements are then known as transposons or jumping genes.

Transposable elements
- Prokaryotes — IS and Tn (Insertion sequences)
- Eukaryotes — Ac and Ds (Activator, Dissocation)

TRANSPOSABLE GENETIC ELEMENTS

I. History / Discovery of Maize Crop :-

Maize crop a staple food crop of the world with a culture heritage

⇒ Cultivation begin atleast 5000 yrs ago in America during the visit of Columbo After that it spread to North to Canada and south to Argentina.

⇒ The native people of North and South America develop many different varities of maize. They had colourful kernels such as blue, yellow, purple, black, white associated with religious values. Some group considered kernels with stripes and spots, is a sign of strength and vigour.

⇒ Modern researches has shown that these stripes or spots on maize are the result of genetic phenomene called transposons. Scientist have found DNA sequences that can move

from one position to another. These elements are called as jumping genes or transposable elements that constitute an appreciable fragment of the genome.

⇒ When transposable element moves from one location to another, they may break or mutate gene. Therefore these elements have great genetic significance. Transposable elements are found in the genome of many organisms such as prokaryotes, eukaryotes (plants, animals, bacteria, humans)

⇒ They are structurally and functionally diverse.

⇒ Role- They clearly have role in shaping the structure of chromosome and in modulating the expression of gene.

Classification on the basis of How they transpose

It is of three types:-

1) Categories
2) Examples

Classification on the basis of how they transpose →

Categories	Examples	Host organism.
Cut and paste transposon	Is elements (Complete)	Bacteria
	Tn elements	"
	Ac/Ds elements	Maize
	Sleeping beauty	Salmon
Replicative transposon	Tn_3	Bacteria
Retrotransposon	F, G, I	Drosophila
	LINE (L_1)	Humans
	SINEs (ALU)	"

Cut and paste transposon → In first category, an element is physically cut out from one site in the chromosome and inserted into another site of the chromosome by an enzyme transcriptase.

Replicative transposon → In the second category, a replicative transposon is copied during the process of transposition. During this process, element is replicated, one copy of it is inserted into new site and one remain at its original site. (Net gain of one copy)

3) Retrotransposon → In the third category, a retro-transposon, produce RNA molecules that are reverse transcribed to DNA molecule with the

Gibberellin makes the amlase active. α-amylase present in the aleurone layer which break the starch into soluble form. (Simple sugar) Simple sugar is taken by scutellum

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help of enzyme reverse transcriptase.

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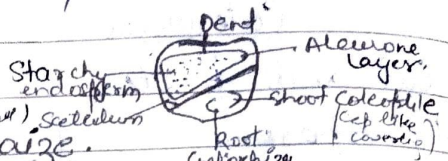
16-09-19
Ozone day



Ac and Ds elements in maize.

The Ac and Ds elements in maize were discovered by an American scientist Barbara McClintock. Barbara showed that the activities of these elements are responsible for striping and spotting of maize kernels.

⇒ Barbara discovered the Ac (Activator) and Ds (Dissociation) element by studying chromosome breakage. She used genetic markers (molecular markers) that control the colours of maize to detect the breakage event. When a particular marker was lost she inferred (observed) that the chromosome segment on which its (marker) was located has also been lost, and it indicates that a breakage event had occur. The loss of a marker was detected by change in the colour of aleurone layer. It is the outermost layer of the triploid endosperm of the maize kernels.

* A genetic marker is a gene or DNA sequence with a known location on chromosome that can be used to identify the individuals or species. It can be described as a variation that can be observed. It may be a short

DNA sequence (single base pair SNP; Single nucleotide polymorphism or a large called as mini satellite). The development of the genetic markers could identify the genetic characteristics.

Type of genetic markers:

1) RFLP (Restriction Fragment Lead Polymorphism)

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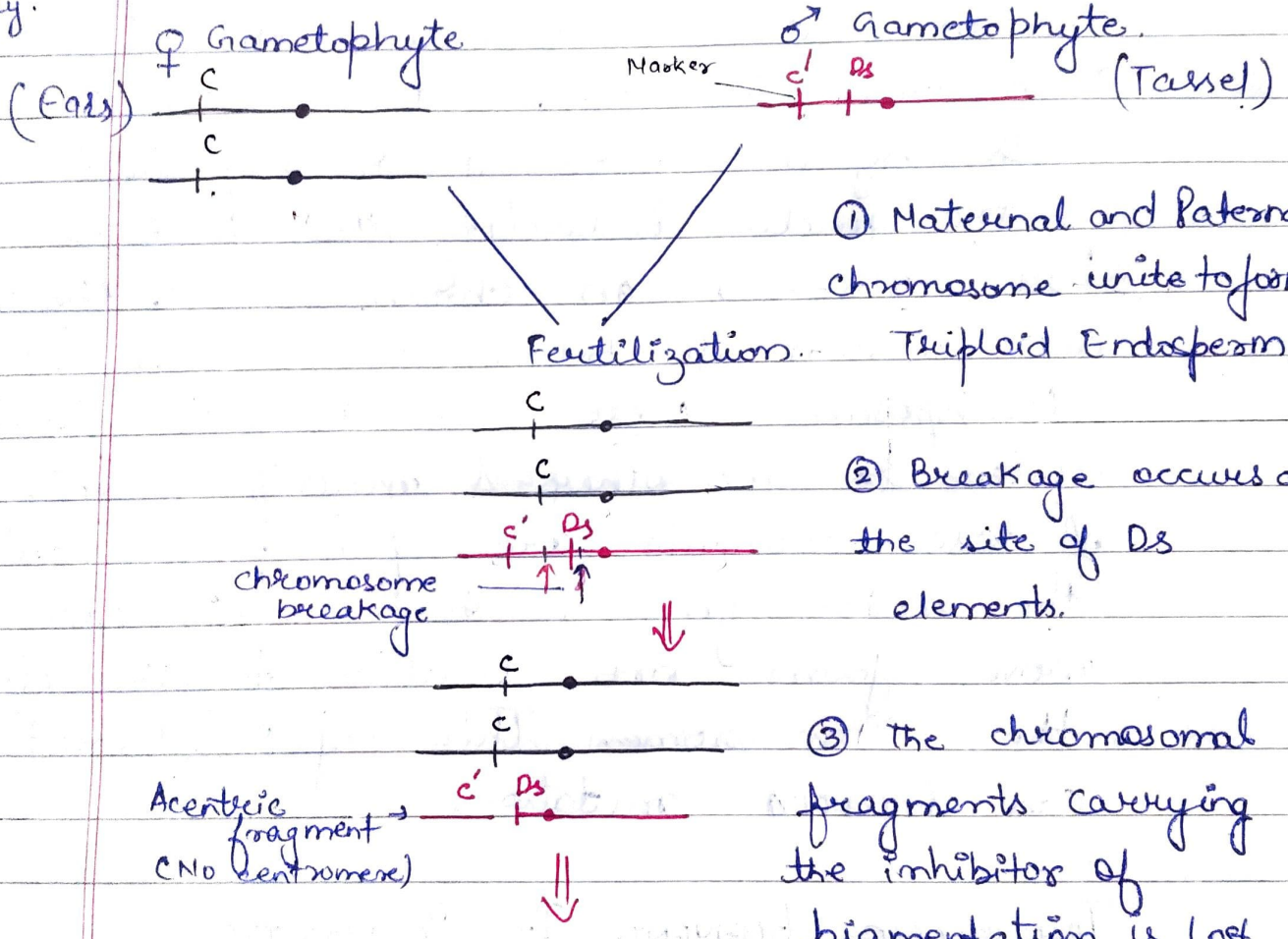
Tuesday.



① Maternal and Paternal chromosome unite to form Triploid Endosperm.

② Breakage occurs at the site of Ds elements.

③ The chromosomal fragments carrying the inhibitor of pigmentation is lost.

④ clone of pigmentation formed.

Clone of pigmented cell (cc)

Barbara found that the breakage responsible for mosaic kernels formed at a particular site on chromosome 9 of maize. She name the factor that produce these breaks called Ds, it means dissociation.

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However, this factor was unable to induce chromosome breakage. This ~~factor~~ was chromosome breakage has to stimulated by another factor called Ac (Actuator). The Ac factor was present in some maize stock but absent in others. When different stock were crossed Ac could be combine with Ds elements and to create the condition that lead to chromosomal breakage. This two factor Ac/Ds system provided an explanation for the genetic instability that Barbara had observed on chromosome 9. The Ac and Ds elements belong to the family of transposon. These elements are structurally related to each other & multiple copies of these elements are often present in the maize genome. Both Ac/Ds can move from one location to other on the chromosome. This Ac/Ds elements can induced mutation.

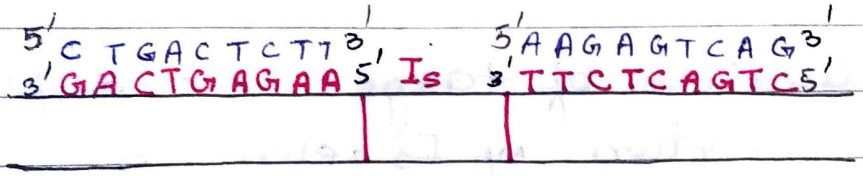
TRANSPOSABLE ELEMENTS IN PROKARYOTES.

Transposable elements were originally discovered in eukaryotes, bacterial transposon were first to be studied. There are 3 main types of

1) Is (Insertion sequence)
2) Tn (Composite transposon)
3) Tn_3

These 3 are differ in size and structure. The Is are the simplest containing gene can encode protein involve in transposition. The other two types are more complex containing some genes that encode proteins unrelated to transposition process.

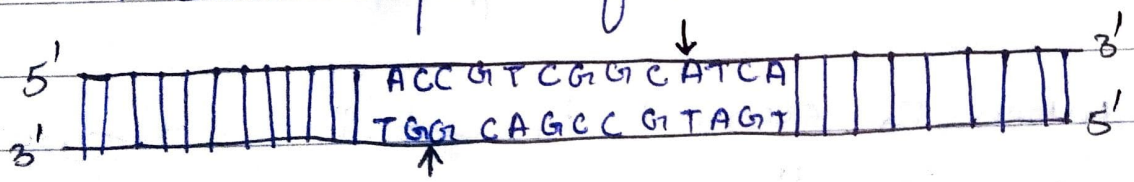
1) Is elements - The simplest bacterial transposon are Is. So named because they can insert at many different sites in bacterial chromosome or plasmid. The Is element was first detected in certain Lac- mutations of E. coli.

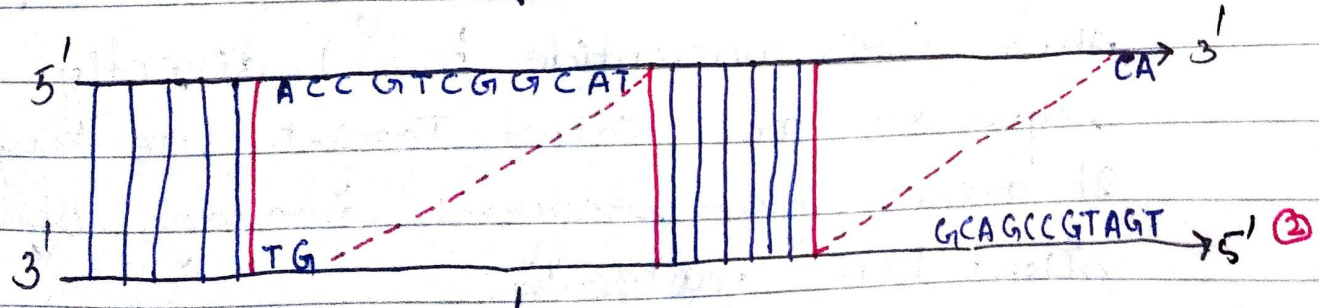


Structure of Is SO elements.

Structure of an inserted Is SO element showing its terminal inverted repeats.

Terminal inverted repeats are imperfect because 4th nucleotide pair from each end is different.





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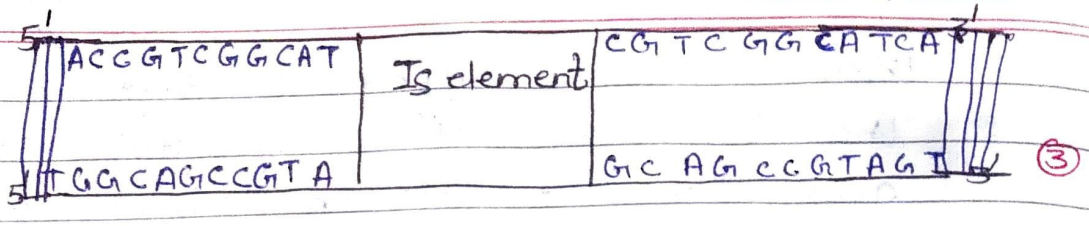

① The two strands of target DNA are cleaved at different locations.

② The Is element is inserted into the gap created by staggered / sticky cleavage of the target DNA.

③ DNA synthesis fill in the gaps on each side producing a direct duplication of target DNA.

④ Production of target site duplication by the insertion of Is elements.

* The Is element usually encode a protein called Transposase which is needed for transposition. The transposase bind at or near the ends of element and then cut both the strands of DNA.

.....

When Is element insert into chromosome or plasmid, they create a duplication of DNA sequences at the site of insertion. These short (2-13 nucleotide pairs) directly repeated sequences are called Target site duplication. It arises from staggered cleavage of double stand DNA molecule.

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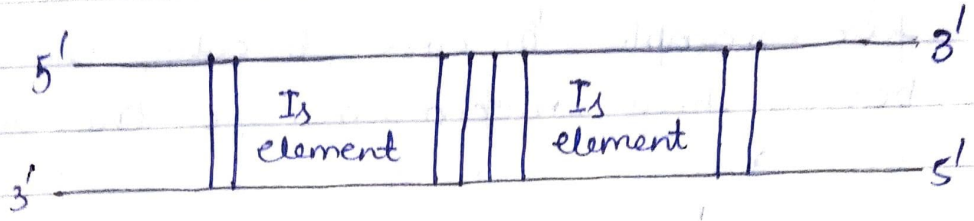
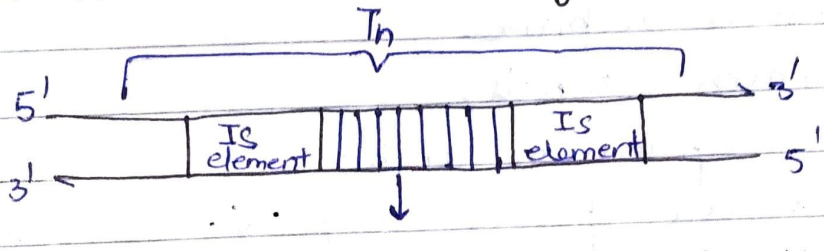
Tn or Composite element

They are created when two Is elements are inserted neare each other. The region between the two Is elements can be transposed when the two elements act jointly.



Tn elements in prokaryotes



Central- move or Transposition due to sequence jointly action of Two Is elements.

Due to the presence of these two Is elements, the DNA sequence present in between the two regions can be transposed.

Eg. Tn_3, is a replicative transposon that transpose by temporary fusion of DNA molecules. Therefore, the fusing DNA molecule form a new copy of Tn_3 element.

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DNA Damage and Repair Mechanism:

Living organisms contain many enzymes that scan their DNA for damage and initate repair process when damage is detected.

The repair mechanism in organism ranging from bacteria to humans for example in case E. coli, the cell posses five mechanism for the repair of damage DNA or defected DNA.

Repair Mechanism in case of E. coli

1) Light dependent repair or Photoreactivation.
2) Excision repair.
3) Mismatch repair.
4) Post replication repair.
5) The error prone repair system. - SOS response

* Mammals possess all the repair mechanisms found in E. coli except photoreactivation because most mammalian cells do not have access to light.

1) Light dependent repair / photoreactivation:

This process in bacteria is carried out by a light activated enzyme called DNA photolyase when DNA is exposed to UV light, thymine dimers are produced by covalent cross linkage between adjacent thymine.

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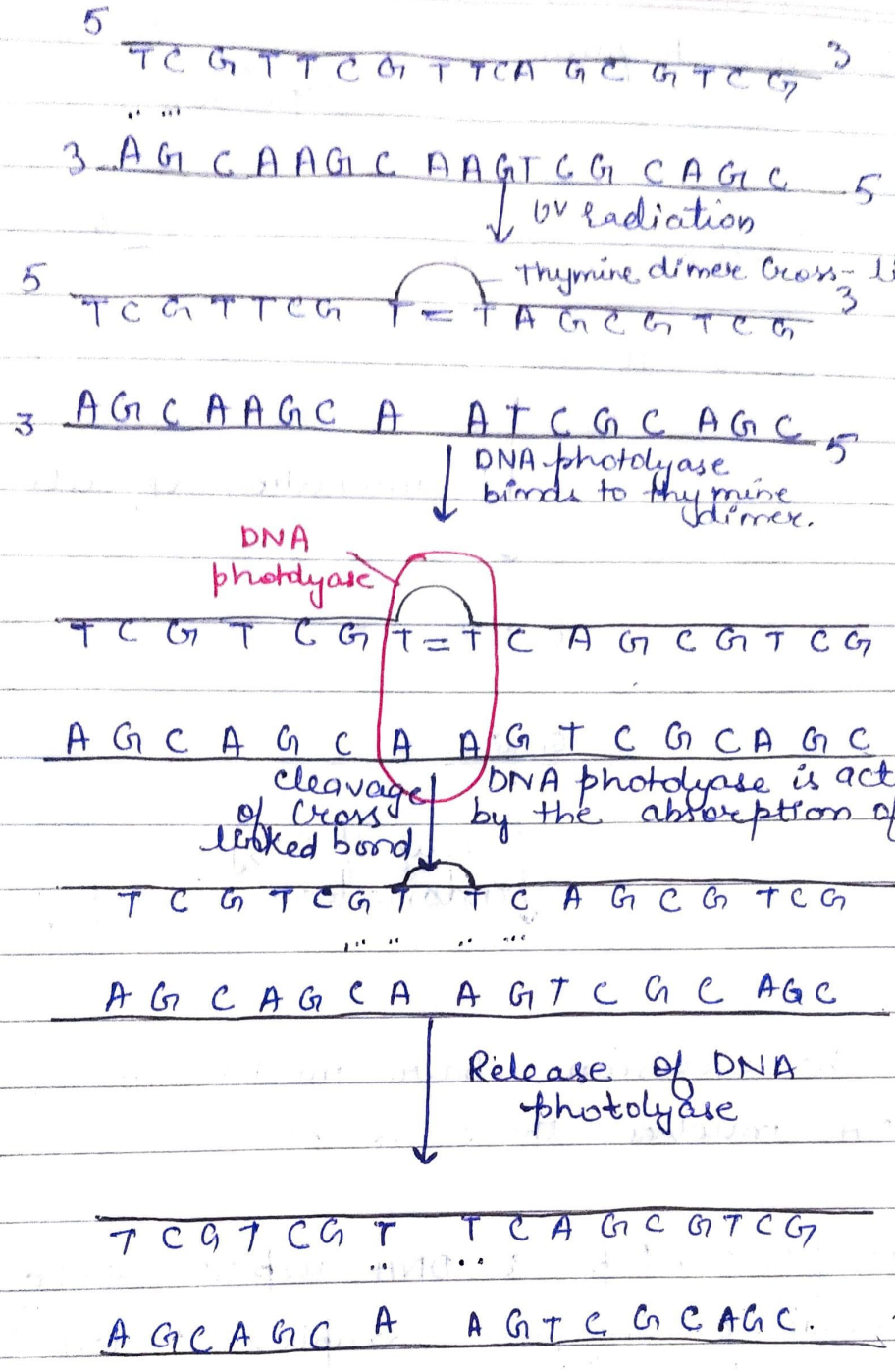

After that DNA photolyase recognises and bind to damage site (thymine dimer) and initiate the process of repair. This DNA photolyase bind to dimer in dark but they are activated thymine by the absorption of blue light.

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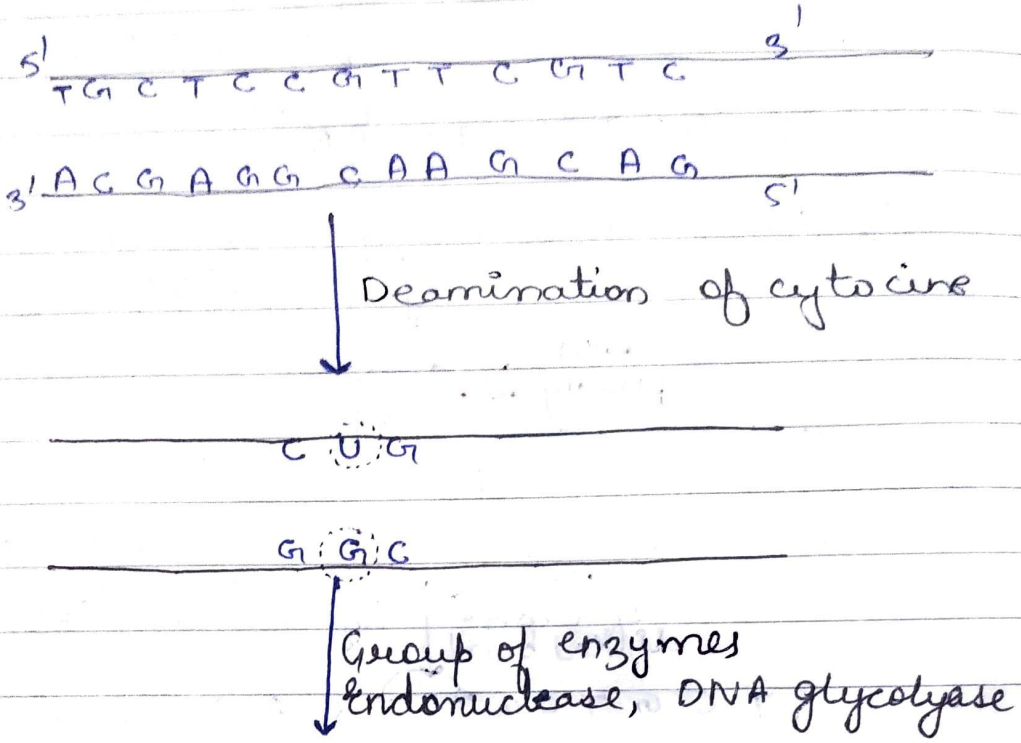
② Excision Repair :-

3 steps ① ② ③



~~Step 1~~: The excision repair mechanism of damaged DNA includes three steps :-

⇒ In first step, a DNA repair endonuclease containing enzyme complex called DNA glycolyases recognise, bind and to cut / excise the damaged base in DNA.

⇒ In second step, a DNA polymerase fill in the gap by using undamaged complementary strand of DNA as template strand.

⇒ In third step, the enzyme DNA ligase seal the break and to complete the repair process.

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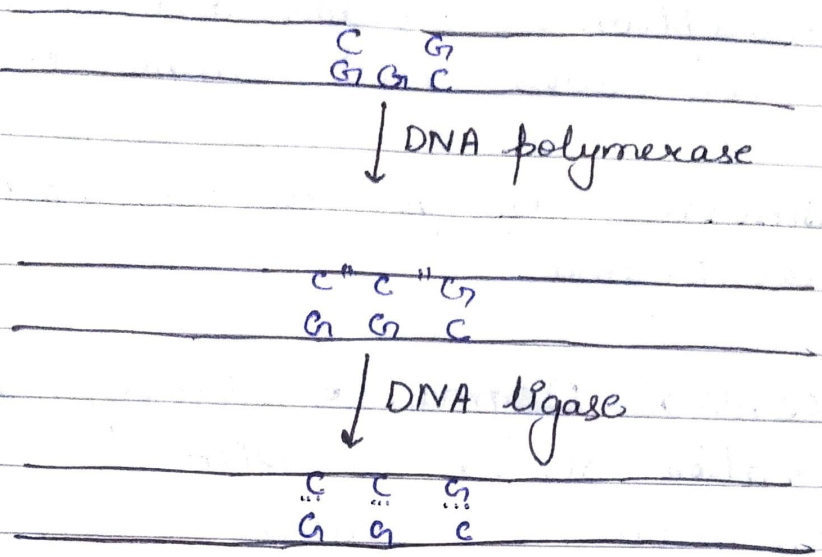


Retrotransposon :-

Transposable elements in Humans :-

44% of Human DNA contain transposable elements including retrovirus like elements and retroposons (33%) and several other transposons families.

The principle transposable element is retrotransposon L_1.

Phloem Loading and Unloading in Plants

Translocation of organic solutes such as **sucrose** (i.e. photosynthetic) takes place through sieve tube elements of phloem from **supply end** (or source) to **consumption end** (or sink).

But, before this translocation of sugars could proceed, the soluble sugars must be transferred from mesophyll cells to sieve tube elements of the respective leaves.

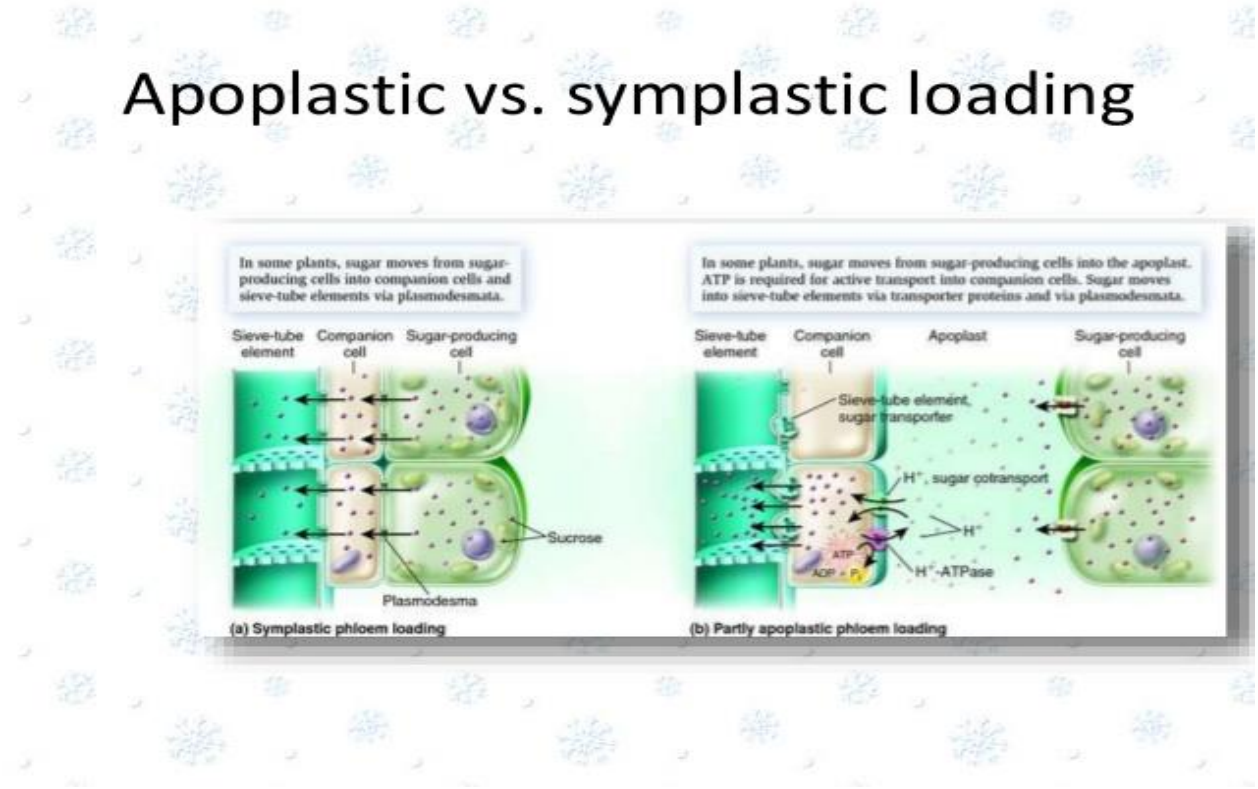
This **transfer** of sugars (photosynthetic) from **mesophyll cells** to **sieve tube** elements in the leaf is called as **phloem loading.**

On the other hand, the transfer of sugars (photosynthetic) from sieve tube elements to the receiver cells of consumption end (i.e., sink organs) is called as **phloem unloading.**

Both are **energy** requiring processes.

As a result of photosynthesis, the sugars such as **sucrose** produced in mesophyll cells move to the sieve tubes of smallest veins of the leaf either directly or through only 2-3 cells depending upon the leaf anatomy. Consequently, the concentration of sugars increases in sieve tubes in comparison to the surrounding mesophyll cells.

The movement of sugars from mesophyll cells to sieve tubes of phloem may occur either through **symplast** (i.e., cell to cell through plasmodesmata, remaining in the cytoplasm) or the sugars may enter the **apoplast** (i.e., cell walls outside the protoplasts) at some point en route to phloem sieve tubes.



Apoplast movement---In this process, sugars are actively loaded from apoplast to sieve tubes by an energy driven transport located in the plasma membrane of these cells.

The mechanism of phloem loading in such case has been called as **sucrose-H^+ symport or cotransport mechanism.**

According to this mechanism -----protons (H^+) are pumped out through the plasma membrane using the energy from **ATP** and an **ATPase** carrier enzyme, so that concentration of **H^+** becomes higher outside (in the apoplast) than inside the cell.

Spontaneous tendency toward equilibrium causes protons to diffuse back into the cytoplasm through plasma membrane coupled with transport of sucrose from apoplast to cytoplasm through sucrose -H^+ symporter located in the plasma membrane.

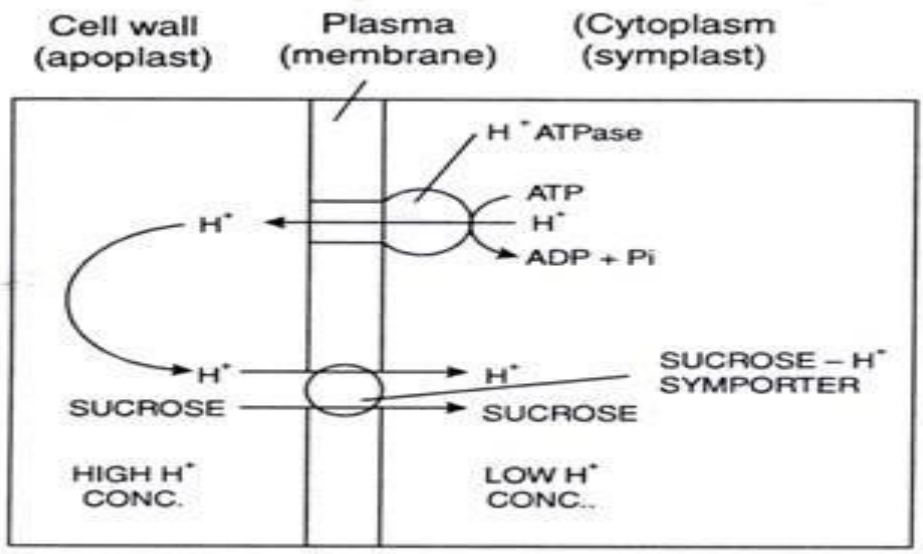


Fig. 15.5. Sucrose –H⁺ symport or cotransport mechanism.

The mechanism of the transfer of sugars (sucrose) from mesophyll cells to apoplast is however, not known.

Phloem loading is specific and selective for transport sugars. Both symplastic and apoplastic pathways of phloem loading are used in plants but in different species.

In some species however, phloem loading may occur through both the pathways in the same sieve tube element or in different sieve tube elements of the same vein or in sieve tubes in veins of different sizes.

Table 15.1 Patterns in apoplastic and symplastic phloem loading.

	Apoplastic loading	Symplastic loading
Type of sugar transported	Sucrose	Sucrose + other oligosaccharides
Type of companion cells in the small veins	Ordinary or transfer cells	Intermediary cells
Number of plasmodesmata connecting the sieve tubes (including companion cells) to surrounding cells	Fewer	Abundant

Phloem Unloading:

It occurs in the consumption end or sinks organs (such as developing roots, tubers, reproductive structures etc.)

Sugars move from sieve tubes to receiver cells in the sink involving following steps:

(i) Sieve element unloading:

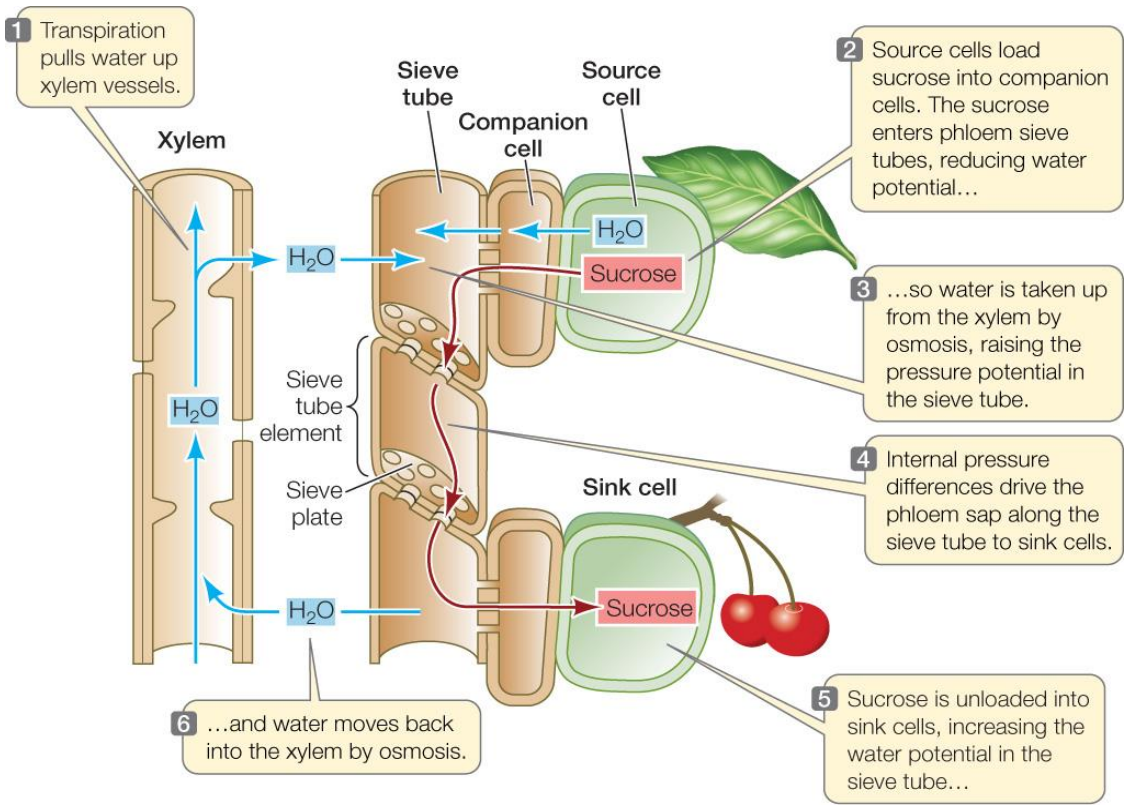
In this process, sugars (imported from the source) leave sieve elements of sink tissues.

(ii) Short distance transport:

The sugars are now transported to cells in sink by a short distance pathway which has also been called as **post-sieve element transport.**

(iii) Storage and metabolism:

Finally, sugars are stored or metabolized in the cells of the sink.



Photorespiration
Or
C2 Cycle
Or
Photosynthetic Carbon Oxidation Cycle (PCO-Cycle)
Or
Glycolytic Metabolism Cycle

The excessive respiration that takes place in green cells in the presence of light is called as **photorespiration.**

Decker (1955) discovered the process and it is also called as **C_2 cycle** as the 2 carbon compound **glycolic acid** acts as the substrate in photorespiration.

In general, respiration takes place under both light and dark conditions. However in some plants, the respiration is more in light than in dark. It is 3-5 times higher than the rate of respiration in dark.

Photorespiration is carried out only in the presence of light. But the normal respiration is not light dependent and it is called dark respiration.

In photorespiration, temperature and oxygen concentration play an important role. Photorespiration is very high when the temperature is between 25 and 30 ºC. The rate of photorespiration increases with the increase in the concentration of oxygen.

Three cell organelles namely ***chloroplast, peroxisome and mitochondria*** are involved in the photorespiration. This kind of respiration is seen in plants like cotton, pulses, capsicum, peas, tomato, petunia soybean, wheat, oats, paddy, chlorella etc and it is absent in grasses.

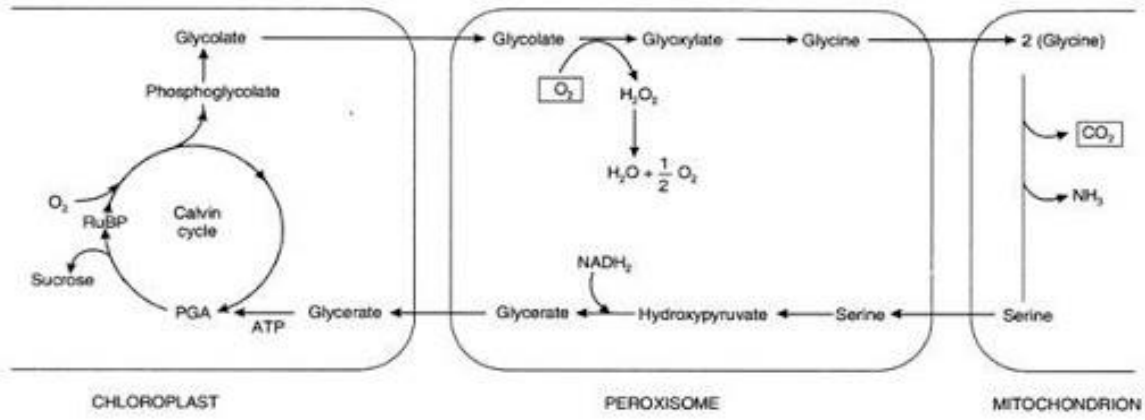
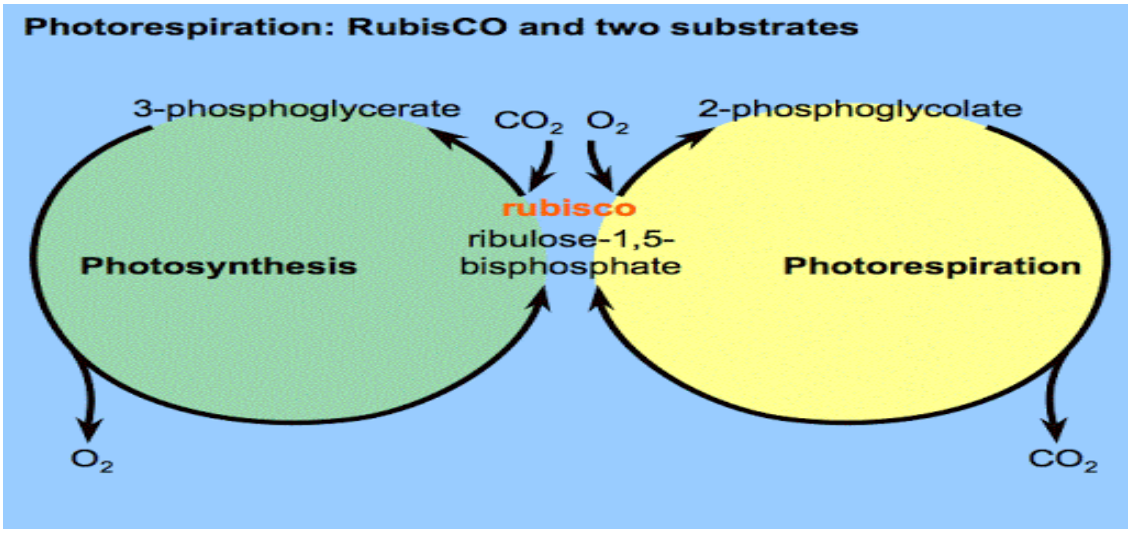


Fig. 11.26. Glycolate metabolism during photorespiration (see text for details).

Mechanism

1. In the presence of excess oxygen and low CO_2 , ribulose 1,5 diphosphate (RuBP) produced in the chloroplast during photosynthesis is split into **2 phospho glycolic** acid and **3 phospho glyceric** acid by the enzyme**, (Rubisco) ribulose 1,5 diphosphate oxygenase.**



2. The 3 phospho glyceric acid enters the Calvin cycle.

3. In the next step, phosphate group is removed from **2 phosphoglycolic acid** to produce **glycolic acid (Glycolate)** by the enzyme, **phosphatase.**

4. Glycolic acid then it come out of chloroplast and enter the peroxisome.

Here, it combines with **oxygen** to form **glyoxylic acid (Glycoxlate)** and hydrogen peroxide. This reaction is catalyzed by the enzyme, **glycolic acid oxidase**.

Hydrogen peroxide (**H_2O_2**) is toxic and it is broken down into **water and oxygen** by the enzyme, **Catalase.**

Photorespiration is an oxidation process. In this process, **glycolic acid** is converted into **carbohydrate** and **CO_2** is released as the by product.

As glycolic acid is oxidized in photorespiration, it is also called as **glycolate metabolism**.

5. The **glyoxylic acid** converted into **glycine** by the addition of **one amino group** with the help of the enzyme, **amino transferase.**

Now, the glycine is transported from the **peroxisome** into the **mitochondria.**

6. In the mitochondria, two molecules of glycine condense to **form serine** and liberate carbon dioxide and ammonia.

7. Amino group is removed from serine to form hydroxyl pyruvic acid in the presence of the enzyme, transaminase.

8. **Hydroxy pyruvic acid** undergoes reduction with the help of NADH to form **glyceric acid** in the presence of enzyme **alpha hydroxyl acid reductase.**

9. Finally, regeneration of **3 phosphoglyceric acid** occurs by the phosphorylation of glyceric acid with **ATP.** This reaction is catalyzed by the enzyme, **Kinase.**

10. The **3 phosphoglyceric acid** is an intermediate product of Calvin cycle.

If it enters the chloroplast, it is converted into carbohydrate by photosynthesis.

Thus, starting from intermediates of Calvin cycle with the synthesis of glycolate, serine is formed which is agin converted into intermediates of Calvin cycle and completing the glycolate cycle.

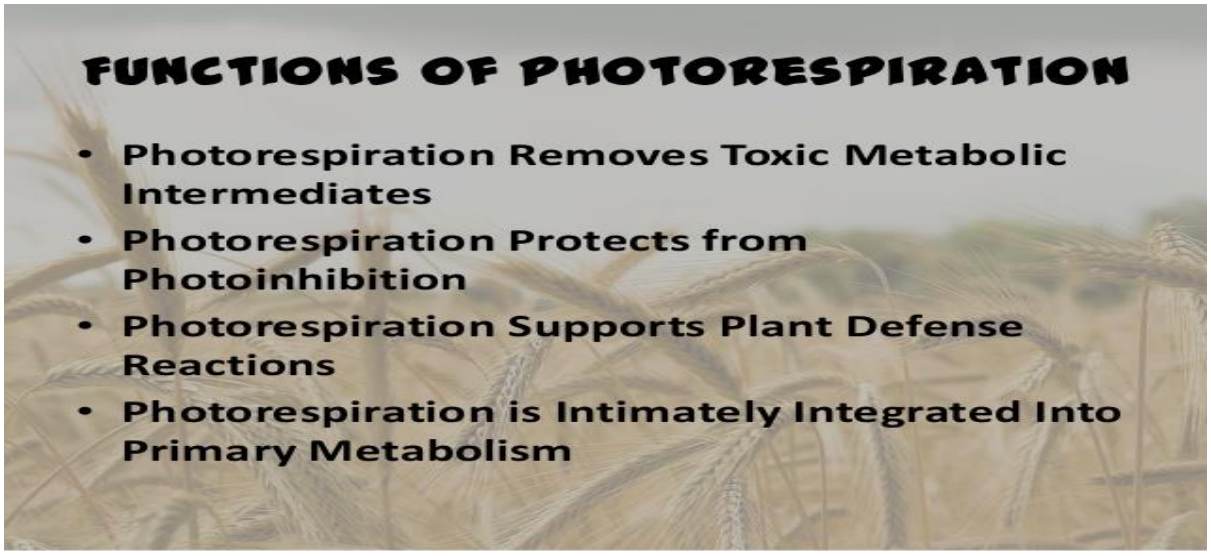
Significance of photorespiration

1. Photorespiration helps in classifying the plants, Generally, photorespiration is found in C_3 plants and absent in C_4 plants.

2. Carbon dioxide is evolved during the process and it prevents the total depletion of CO2 in the vicinity of chloroplasts

3. Photorespiration uses energy in the form of ATP and reduced nucleotides, but normal respiration yields ATP and reduced nucleotides.

4. It is believed that photorespiration was common in earlier days when CO2 content was too low to allow higher rates.



Anil Kumar Dogra

Assistant Professor (Botany)

Red Drop and Emerson's Enhancement Effect

Robert Emerson noticed a sharp decrease in quantum yield at wavelength greater than **680 nm**, while determining the quantum yield of photosynthesis in **Chlorella** using monochromatic light of different wavelengths. Since this decrease in quantum yield took place in the red part of the spectrum, the phenomenon was called as **Red Drop.**

Later, they found that the inefficient far-red light beyond 680 nm could be made fully efficient if supplemented with light of **shorter wavelength.** The quantum yield from the **two** combined beams of light was found to be **greater than** the sum effects of both beams used separately. This enhancement of photosynthesis is called as **Emerson's Enhancement.**

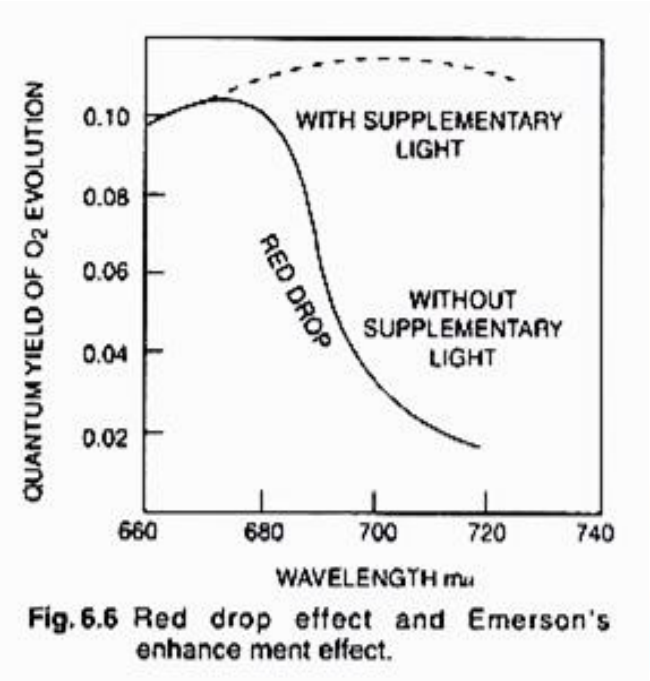


Fig. 6.6 Red drop effect and Emerson's enhance ment effect.

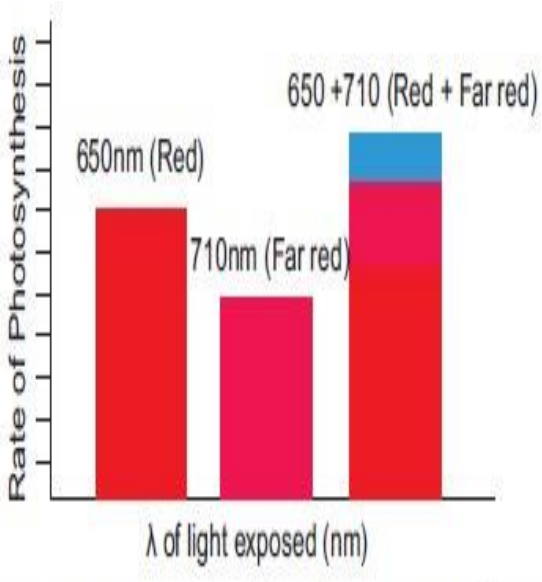
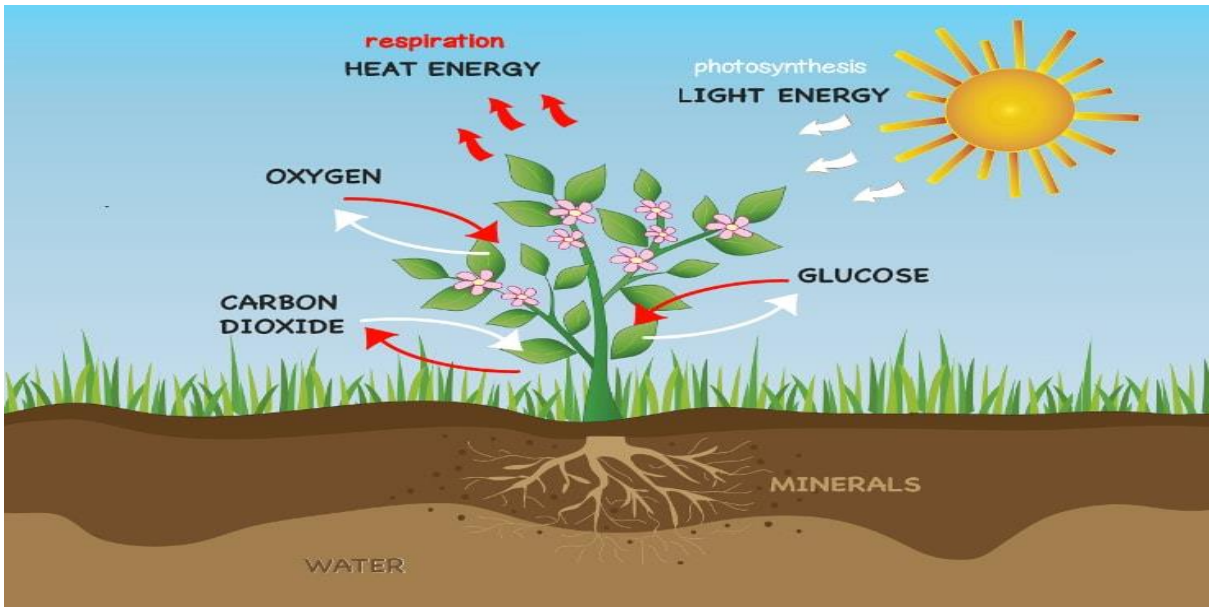


Figure 13.9: Emerson's Enhancement Effect

RESPIRATION IN PLANTS



The cellular oxidation or break down of carbohydrates into CO_2 and H_2O and release of energy is called as **respiration.**

It is a **reverse** process of photosynthesis.

In respiration, the oxidation of various organic food substances like **carbohydrates, fats, proteins** etc, may take place. Among these, **glucose** is the commonest.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{Energy (686 kcal)}$$

This oxidation process in not so **simple** and does not take place in one step. Breakdown of glucose involves many steps releasing energy in the form of **ATP** molecules and also forming a number of carbon compounds (intermediates).

Respiration is a vital process that occurs in all living cells of the plant and the most actively respiring regions are floral buds, vegetative buds, germinating seedlings, stem and root apices.

Types of Respiration

Degradation of organic food for the purpose of releasing energy can occur with or without the participation of oxygen.

Hence, respiration can be classified into two types:

1. Aerobic Respiration

2. Anaerobic Respiration

1. Aerobic Respiration

Aerobic respiration takes place in the presence of **oxygen** and the respiratory substrate gets completely oxidized to carbon dioxide and water as end products.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + \text{Energy (686 kcal)}$$

Glucose

2. Anaerobic Respiration

It takes place in the absence of **oxygen** and the respiratory substrate is incompletely oxidized. Some other compounds are also formed in addition to carbon dioxide. This type of respiration is of rare occurrence but, common among microorganisms like yeasts.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 + 56 \text{ kcal}$$

Glucose Ethanol

Respiratory Substrate

A respiratory substrate is an organic substance which can be degraded to produce energy which is required for various activities of the cell. The respiratory substrates include **carbohydrates, fats, organic acids, protein** etc.

Carbohydrates: The carbohydrates constitute the most important respiratory substrate and the common amongst them are starch, sucrose, glucose and fructose. The complex carbohydrates are first hydrolyzed to simple sugars and then they are utilized.

$$\text{Starch} \rightarrow \text{Disaccharides} \rightarrow \text{Hexoses}$$

Fats: The fats are important storage food in seeds. Nearly 80 per cent of the angiosperms have fats as the main storage food in their seeds. At the time of seed germination, large amount of fats are converted into carbohydrates while the remaining fats are utilized in respiration.

Fats are first broken down to **glycerol** and **fatty acids**. The fatty acids are broken down to **acetyl coenzyme** by **β-oxidation**. The acetyl coenzyme enters **Kreb's cycle** for further degradation and releases energy. Glycerol can directly enter the respiratory channel via glyceraldehyde.

Organic acids: Organic acids normally do not accumulate in plants to any appreciable extent except in the members of the family, Crassulaceae. Organic acids are oxidized under aerobic conditions to carbon dioxide and water.

Proteins: Under normal conditions, proteins are used up as respiratory substrate only in seeds rich in storage proteins. In vegetative tissues, proteins are consumed only under

starvation. The proteins are hydrolyzed to form amino acids. Later, the amino acids undergo deamination forming organic acids and the organic acids can enter Kreb's cycle directly.

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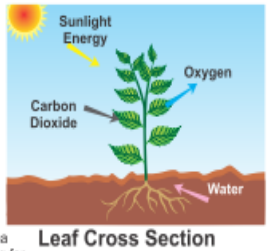
Respiration and Transpiration in Plants

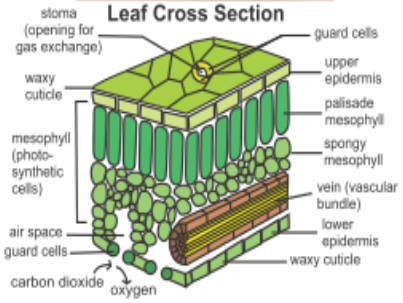
All parts of the plant respire, but the main respiratory organ of plant is leaf. Gases like oxygen and carbon dioxide enters leaf through **stomata**.

During day time photosynthesis and respiration take place simultaneously, however photosynthesis is the more vigorous of the two processes.

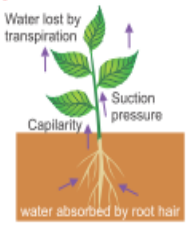
During day time, photosynthesis take place in leaf. Carbon dioxide from the atmosphere is taken in and oxygen diffuses out of the leaf through stomata.

During night, photosynthesis does not take place. Oxygen diffuses through the stomata into the leaf carbon dioxide and water vapour are given out.





Transpiration in Plants



The process of **evaporation of excess water from leaves** is called transpiration.

Advantages of transpiration

As water transpires more water is drawn from the xylem. This exerts pressure and more water is sucked in through the roots. This helps to lower and main temperature of the plant.

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Mechanism of Respiration

1. Glycolysis

2. Aerobic breakdown of pyruvic acid (Kreb's cycle)

3. Electron Transport System/ Terminal oxidation / oxidative phosphorylation

4. Pentose phosphate pathway.

Rice: Origin and Cultivation

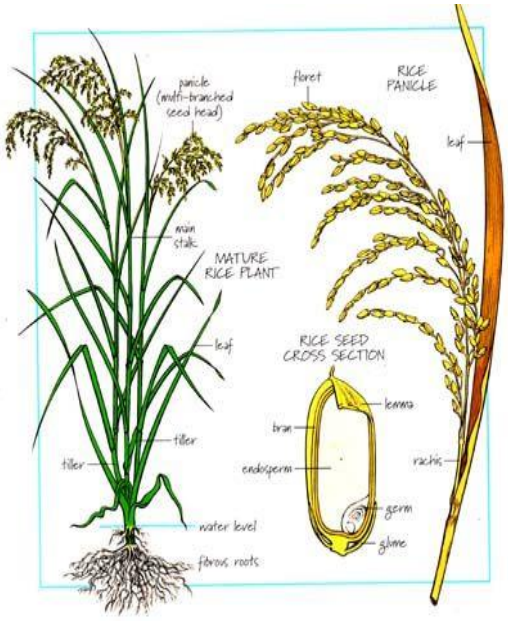
Rice (*Oryza sativa* L.) is a plant belonging to the family of grasses, Gramineae (Poaceae). It is one of the three major food crops of the world and forms the staple diet of about half of the world's population. Asia is leading in rice production accounting for about 90% of the world's production.

India has a long history of rice cultivation. Globally, it stands first in rice area and second in rice production, after China. It contributes 21.5% of global rice production. India is one of the leading exporter of rice, particularly basmati rice.

Origin

The centre of origin and centres of diversity of two cultivated species O. sativa and O. glaberrima have been identified using genetic diversity, historical and archaeological evidences and geographical distribution. It is generally agreed that river valleys of Yangtze, Mekon rivers could be the primary centres of origin of O. sativa while Delta of Niger River in Africa as the primary centre of origin of O. glaberrima.

The foothills of the Himalayas, Chhattisgarh, Orissa, north-eastern India, northern parts of Myanmar and Thailand, Yunnan Province of China etc., are some of the centres.



Morphology

Rice is a monocarpic plant that flowers once, set seeds and then die. Cultivated rice plant is an annual grass growing to 1–1.8 m with round, hallows and jointed culms, flat leaves and a terminal inflorescences called panicle. Each culm or tiller is a shoot, which includes root, stem and leaves.

Root

The root system consists of two major types:

Crown roots (the adventitious roots, including mat roots) that develop from nodes below the soil surface.

The primary (seminal) roots-----Primary root is direct prolongation of radicle that usually dies within a month.

The main rooting system of the plant develops at later stages of plant growth, when roots develop horizontally from the nodes of the stem below ground level (crown roots).

Stem

The stem has 2 parts------underground and aerial.

The aerial part, has well defined solid nodes and hollow internodes. Another name for the aerial part of the stem of the rice plant is the culm which consists of several nodes spaced apart by internodes.

The culm is more or less erect, cylindrical, and hollow except at the nodes.

The panicle also forms at the uppermost node and gives rise to the spikelets or fruits of the rice plant.

Leaf

Leaves on the main stem are produced one at a time and are arranged alternatively. The number of leaves borne on an axis is equal to the number of nodes.

The first leaf of the plant is the sheathing leaf or coleoptile. The second leaf emerging through the lateral sheath of the coleoptile is reduced in size and has no blade.

The remaining leaves are normal, except the uppermost or "flag" which is slightly modified, its major function is to perform photosynthesis.

Flowers

Inflorescence of rice is a terminal panicle (compound raceme) with single flowered spikelets, born on a long peduncle, which is the last internode of the culm.

Panicle formation occurs at the tip of the growing point of the shoot.

Grain

Rice grain, a caryopsis, is a dry one seeded fruit having its pericarp fused with seed coat. The endosperm is made of starch, protein and fat. The endosperm consists of aleurone layer that encloses the embryo and the starchy or inner endosperm. It is the storehouse of food for embryo.

Cultivation of Rice

Climate

Rice is grown under varied ecosystems on a variety of soils under varying climatic conditions. The climatic factors affecting the cultivation of rice are:

Rainfall: Rainfall is the most important weather element for successful cultivation of rice. The distribution of rainfall in different regions of the country is greatly influenced by the physical features of the terrain, the situation of the mountains and plateau.

Temperature: Rice being a tropical and sub-tropical plant, requires a fairly high temperature, ranging from 20° to 40°C. The optimum temperature of 30°C during day time and 20°C during night time seems to be more favourable for the development and growth of rice crop.

Soil: The most important group of soils for successful rice cultivation include alluvial soils, red soils, laterite soils and black soils. It is grown normally in soils with soil reaction ranging from 5 to 8 pH.

Season for Rice Crop

The chief rice growing season is ‘kharif’ season also called ‘winter rice’. The sowing time is June-July and is harvested during November- December months. 84% of the country’s rice supply is grown in the kharif crop.

Rice cultivated during rabi season is also called as ‘summer rice’. It is sown in the months of November to February and harvested during March to June. 9% of total rice crop is grown in this season. Early maturing varieties are normally grown during this time.

The pre-kharif or ‘autumn rice’ is sown during May to August. The sowing time also depends on the rainfall and weather condition. Hence the timing may differ slightly from place to place.

Generally, it is harvested during September- October months. 7% of the total rice crop in India grows in this season and short duration varieties which mature within 90-110 days are cultivated.

Rice Cultivation Method

Most farmers practice nursery bed method. Nursery beds are made occupying about 1/20th of the total field area. The paddy seeds are sown in the bed. They are ready within 25 days of sowing in low land areas while in higher altitudes they take about 55 days to become ready for transplantation. There are four different practices of cultivation of rice, viz. transplantation method, drilling method, broadcast method and Japanese method.

Transplantation is the most commonly used method wherein seeds are first sown in nursery and the seedlings are transplanted to the main field once they show 3-4 leaves. Although this is the best yielding method, it requires heavy labour.

Drilling method is exclusive to India. In this method, one person ploughs a hole in the land and the other person sows the seed. Ox is the most commonly used ‘person’ to plough the land.

Broadcast method generally involves scattering of the seeds manually over a large area or in the entire field. Labour involved is very less and so is the precision. This method produces very less yield as compared to others.

Japanese method has been adopted for the high yielding variety of rice and those that need a high amount of fertilizers. Seeds are sown in nursery beds and then transplanted to the main field. It has shown tremendous success for the high yielding varieties.
Another newly found technique is System of Rice Intensification SRI method of rice cultivation. This is a high yielding method with less water but this method is more laborious.

Harvesting

One of the essential factors in rice cultivation is in-time rice harvesting otherwise the grains would shed. Irrigation of the field is completely stopped about a week before harvesting. This dehydration process helps in grain ripening. It also hastens maturity. In case of early and medium maturing varieties, harvesting should be carried out 25- 30 days after flowering. The late maturing varieties are harvested 40 days after flowering. They are generally harvested when the moisture content is about 25%. Post harvesting, drying is carried out gradually under shade.

Utilization

The harvested rice kernel, known as paddy, or rough, rice, is enclosed by the hull, or husk. Milling usually removes both the hull and bran layers of the kernel, and a coating of glucose and talc is sometimes applied to give the kernel a glossy finish.
Rice that is processed to remove only the husks, called brown rice, contains about 8 percent protein and small amounts of fats and is a source of thiamine, niacin, riboflavin, iron, and calcium.
Rice that is milled to remove the bran as well is called white rice and is greatly diminished in nutrients. When white rice forms a major portion of the diet, there is a risk of beriberi, a disease resulting from a deficiency of thiamine and minerals. Parboiled white rice is processed before milling to retain most of the nutrients, and enriched rice has iron and B vitamins added to it.
Rice is cooked by boiling. It is eaten alone and in a great variety of soups, side dishes, and main dishes in Oriental, Middle Eastern, and many other cuisines.

Conclusion

Cultivating rice is indeed laborious and it needs a lot of water. Therefore, rice cultivation is practiced in those places wherein the labour cost is less and rainfall is high.

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GDC Hiranagar

Transgenic Plants or Genetically Modified Plants

terms to know

TRANSGENE- It is a foreign gene or genetic material that has been transferred naturally or by any of a number of genetic engineering techniques from one organism to another.

TRANSGENESIS- The phenomenon of introduction of exogenous DNA into the genome to create and maintain a stable and heritable character.

TRANSGENIC PLANTS- The plant whose genome is altered by adding one or more transgenes are known as transgenic plants.

Transgenic plants are those plants in which their **DNA** is modified using genetic engineering techniques. The aim is to introduce a new trait or gene to the plant which does not occur naturally in the species.

A transgenic plant contains a gene of interest or genes that have been artificially inserted. The inserted gene sequence is known as the **transgene.**

The purpose of inserting a combination of genes in a plant, is to make it

as **useful and productive.**

The first transgenic plants were reported in **1983**. Since then, many recombinant proteins have been expressed in several important agronomic species of plants including tobacco, corn, tomato, potato, banana, alfalfa and canola.

Development of Transgenic Crops

Genetically engineered plants are generated in a laboratory by altering the **genetic-make-up,** usually by adding one or more genes of a plant's genome.

The nucleus of the plant-cell is the target for the new transgenic DNA.

Methods

1. **Biolistic method (Particle gun method)**
2. ***Agrobacterium Tumefaciens* mediated trans- formation method**.

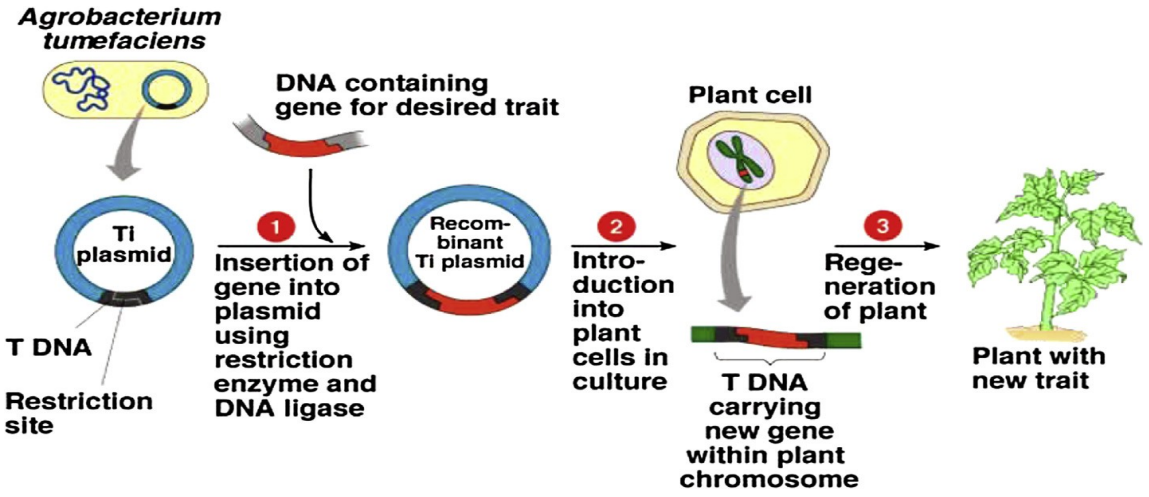
1. Biolistic method (Particle gun method):

The "Gene Gun" method, also known as the **"Micro-Projectile Bombardment**" or "**Biolistic"** method is most commonly used in the species like corn and rice.

In this method, DNA is bound to the tiny particles of Gold or Tungsten, which is subsequently shot into plant tissue or single plant cells, under high pressure using gun.

2. Agrobacterium Tumefaciens mediated trans- formation method:

This method has been applied successfully for many crops, especially monocots, like wheat or maize, for which transformation using **Agrobacterium tumefaciens** has been less successful. This technique is clean and safe. The only disadvantage of this process is that serious damage can be happened to the cellular tissue.



Agrobacterium mediated transformation.

Advantages of transgenic plants

GM Technology has been used to produce a variety of crop plants to date. As the global population continues to expand, food remains a scare resource. Genetically engineered foods offer significant benefits by improving production yield, lowering transportation costs and enhancing the nutritional content.

Several GM crops for malnutrition are expected to be revealed for cultivation in the coming five to ten years.

- Advantages like improving shelf life, higher yield, improved quality, pest resistance, tolerant to heat, cold and drought resistance, against a variety of biotic and abiotic stresses.
- Transgenic plants can also be produced in such a way that they express foreign proteins with industrial and pharmaceutical value

Disadvantages of transgenic crops

The use of transgenic crops was an issue for many years. Many concerns have been raised and these are falling into two categories.

- A concern, about what affect genetically modified material, could have on human health. For example, transgenic crops have been suggested to cause allergies in some people, although it is uncertain, whether transgenic crops are the source of this reaction.
- A concern, about whether transgenic crops cause damage to the

natural environment. One example that includes pollen from transgenic corn, which has capacity to kill the Monarch butterfly larvae.

Should we use transgenic crops?

The perceived advantages and disadvantages of transgenic crops must be married to each other, to provide a crop that is environmentally sound and non-hazardous.

At the least, most would agree that, the potential advantage of producing crops, which provide the human population with more and cheaper food, makes transgenic technology a useful invention.

The future

Although genetically modified crops offer a potential solution to food shortages around the globe, the viability of their cultivation remains questionable. The enhanced production of GM crops to eliminate hunger, carries hidden costs in environment and health concerns.

The issue continues to be controversial and the future of genetically modified crops remains uncertain.

Conclusion

In the future, researchers hope to be able to provide vaccinations and medicines in GM foods, which can provide medications to people in developing countries more easily.

The advancements made with transgenic plants have and will continue to have a great impact on the lives of many.

Examples of Transgenic plants

TABLE 50.10 Some examples of transgenic crop plants (GM plants) at the developmental stages

Plant	*Gene transfer*	*Trait transferred/application(s)*
For improving human health		
Tomato	Phytoene desaturase	Provitamin A (β-carotene) supplement
Canola	γ-Tocopherol methyl transferase	Vitamin E supplement
Sugar beet	Sucrose-sucrose fructosyl transferase	Fructans-low calorie alternatives to sucrose
Rice	Ferritin	Iron supplement
Potato	Antisense threonine synthase	Increased methionine levels
Potato	Seed albumin	Protein with all essential amino acids
Tomato	S-Adenosylmethionine decarboxylase	Increased lycopene levels
Tomato	Chalcone isomerase	Flavanols-act as antioxidants, reduce risk of cancer, heart diseases
Arabidopsis	Isoflavone synthase	Isoflavones-reduce serum cholesterol, and reduce osteoporosis
Canola	Modified acyl-acyl carrier protein thioesterase	*cis*-Stearates-lower the risk of heart diseases
For increased crop yield		
Rice	Phosphoenol pyruvate carboxylase	Increased efficiency of photosynthesis
Tobacco	Phytochrome A	Avoids shades
Lettuce	Gibberellic acid (GA) oxidase	Inhibits GA accumulation and stem growth (dwarfing)
Potato	Phytochrome B	Increased photosynthesis and longer life span
Others		
Tobacco and soybean	Cytochrome P_{450}	Synthesis of epoxy fatty acids for manufacture of adhesives and paints
Rice	Nicotianamine aminotransferase	Tolerance to low iron availability
Tobacco	Nitroreductase	Reduces land contamination by trinitrotoluene

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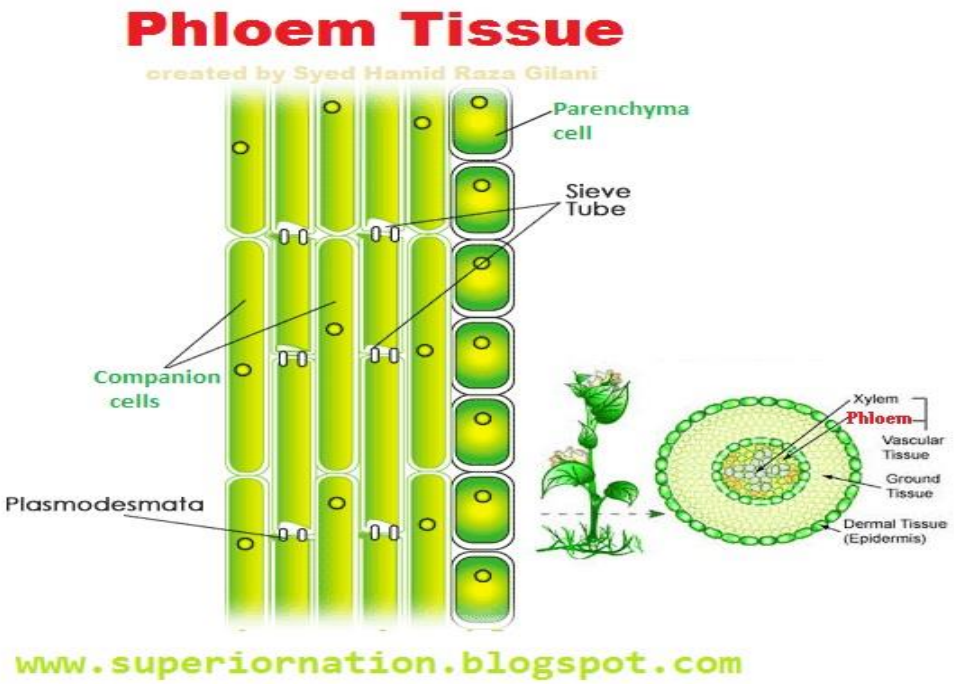
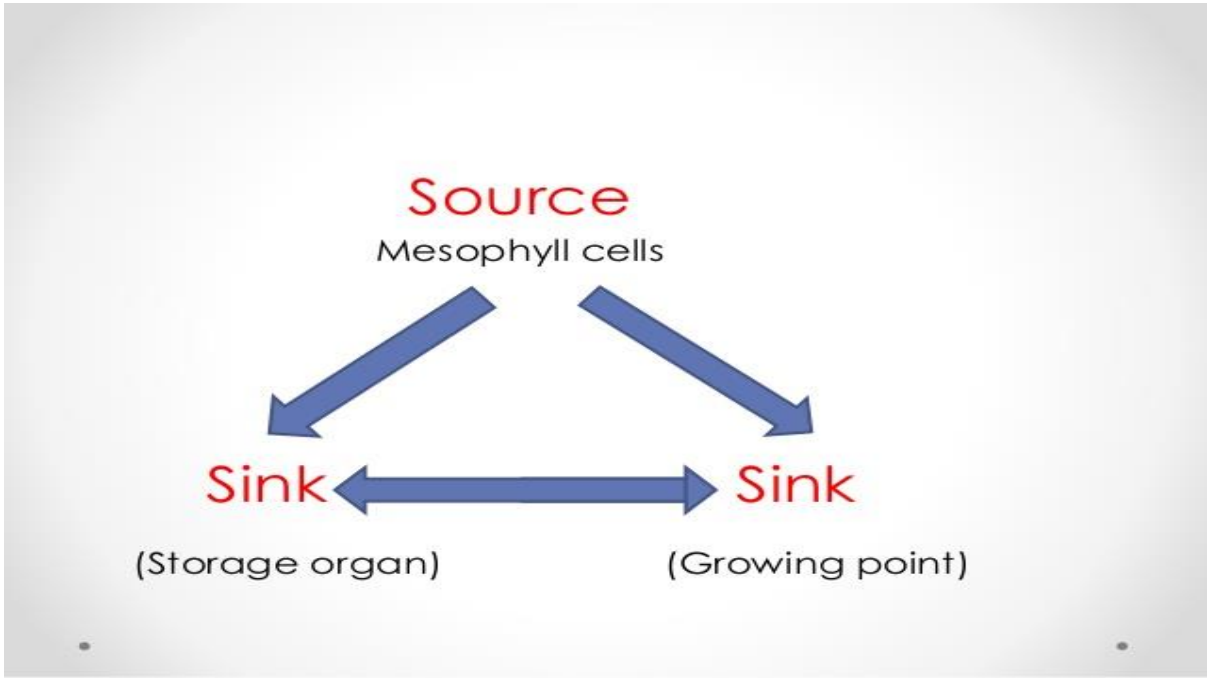
Translocation in Phloem: Girdling Experiment and Pressure Flow Model



Translocation in phloem is the long distance movement of organic substances from the source or supply end (region of manufacture or storage) to the region of utilization or sink. But the source and sink may be reversed depending on the season or need of the plants.



Sugar stored in roots may be mobilised to become a source of food in the early spring when the buds of trees act as **sink** and require energy for their growth and development. Since the source-sink relationship is variable, the direction of movement of organic solutes in phloem can be upwards or downwards i.e., bidirectional.

Directions of Translocation of Organic Solutes:

Translocation of organic solutes can occur in the following directions:

1. Downward Translocation:

It is the most common mode of translocation. The leaves manufacture food in excess of their own requirement. The excess food comes out of leaves and is trans-located in the downward direction to stem (for storage, metabolism, maintenance of its cells and secondary growth, if any) and root system (for storage, growth, metabolism and maintenance).

2. Upward Translocation:

In deciduous plants renewal of growth and development of new foliage are dependent upon upward transport of food from reserves present in the roots and stems. Growth of the stem apices, formation of flowers, fruits, etc. require the movement of assimilates from leaves in an upward direction.

3. Lateral Translocation:

It is little except when source and sink lie on the opposite sides.

4. Bidirectional Translocation:

Rabideau and Burr (1945) found that labelled carbohydrates moved out of the leaves in both upward and downward directions. The two types of translocation are believed by many workers to occur in different sieve tubes.

Pathway of Translocation:

The most common organic nutrient trans-located in plants is **sucrose.** The channels of transport are sieve tubes (in flowering plants) and sieve cells (in non-flowering vascular plants) of phloem. It was proved for the first time by **Czapek (1897).**

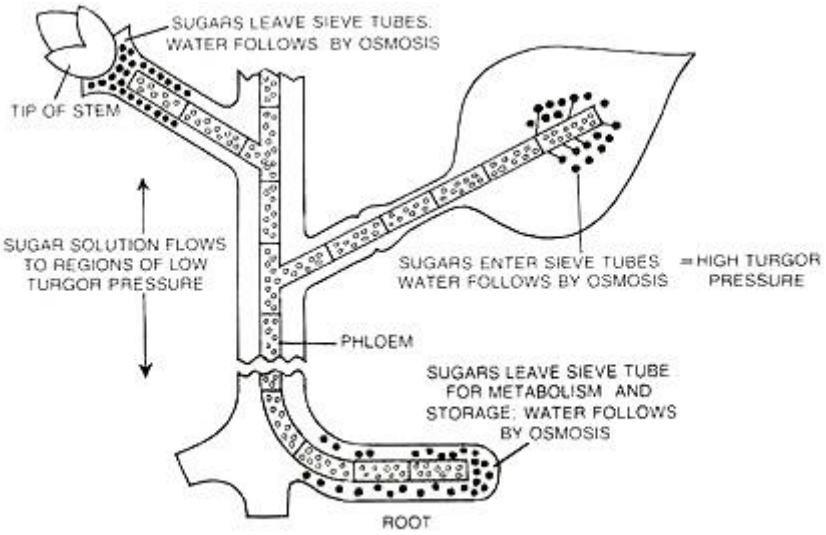


Fig. 11.41. Pathway and mechanism of phloem translocation.

The evidences are as follows:

1. There are only two paths for long distance translocation, tracheary elements and sieve tubes. The former are dead while the latter are living. Translocation of organic solutes seems to be through sieve tubes because it is inhibited by steam girdling which kills living cells.

Girdling or Ringing Experiment

2. In girdling or ringing experiment, a **ring of bark** is **cut** from the stem. It also removes **phloem.** Nutrients collect above the ring where the bark also swells up and may give rise to adventitious roots. Growth is also vigorous above the ring.

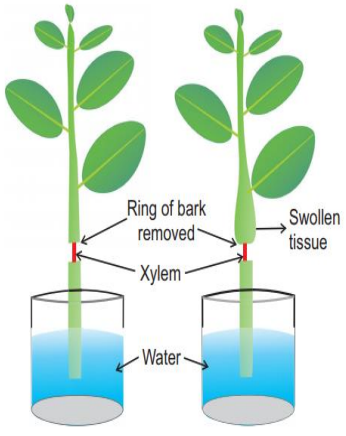


Figure 11.20: Ringing experiment

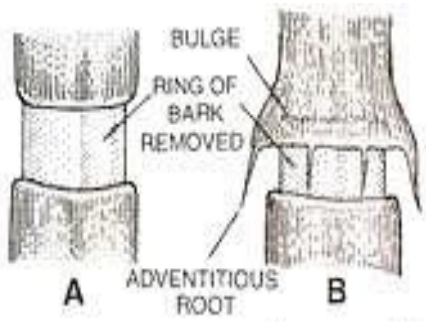


Fig. 11.40. Girdling of tree trunk to show that organic nutrients accumulate in the bark above the girdle where a bulge is also produced.

The tissues below the ring not only show stoppage of growth but also begin to shrivel (Roots can be starved and killed if the ring is not healed after some time. Killing of roots shall kill the whole plant, clearly showing that bark or phloem is involved in the movement of organic solutes which occurs in one direction, i.e., towards root.

Girdling experiments are performed in fruit trees to make more food available to fruits. However, the rings are kept narrow and cambium is not touched so that the incision heals up after some time.

(Girdling experiments cannot be carried out in monocots and dicots with bi-collateral bundles because of the absence of a single strip of phloem).

Mechanism of Phloem Translocation:

Several theories have been put forward to explain the mechanism of translocation of organic nutrients through the phloem e.g., diffusion, activated diffusion, protoplasmic streaming, interfacial flow, elect osmosis, trans cellular strands, contractile proteins, mass flow.

Mass flow hypothesis is the most accepted one.

Mass Flow or Pressure Flow Hypothesis:

It was put forward by **Munch (1927, 1930).** According to this hypothesis, organic substances move from the region of **high osmotic pressure** to the **region of low osmotic pressure** in a mass flow due to the development of a gradient of turgor pressure.

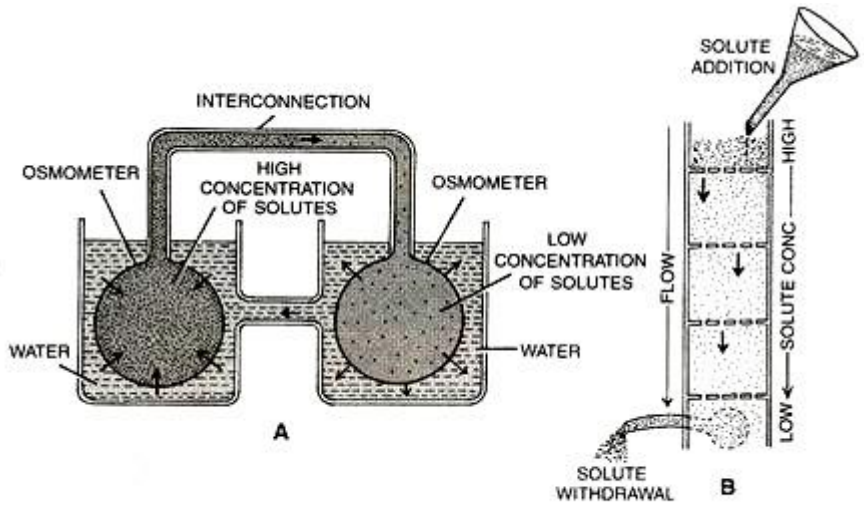


Fig. 11.42. A, mass flow or pressure flow of fluids from high to lower osmotic or turgor pressure. B, diagrammatic representation of continuous flow of solutes in one direction.

This can be proved by taking two interconnected osmometers, one with high solute concentration and the other with little osmotic concentration.

The two osmometers of the apparatus are placed in water. More water enters the osmometer having high solute concentration as compared to the other.

It will, therefore, come to have high turgor pressure which forces the solution to pass into the second osmometer by a mass flow.

If the solutes are replenished in the donor osmometer and immobilised in the recipient osmometer, the mass flow can be maintained indefinitely.

Sieve tube system is fully adapted to mass flow of solutes. Here the vacuoles are fully permeable because of the absence of tonoplast. A continuous high osmotic concentration is present in the source or supply region, e.g., mesophyll cells (due to photosynthesis).

The organic substances present in them are passed into the sieve tubes through their companion cells by an active process.

A high osmotic concentration, therefore, develops in the sieve tubes of the source. The sieve tubes absorb water from the surrounding xylem and develop a high turgor pressure.

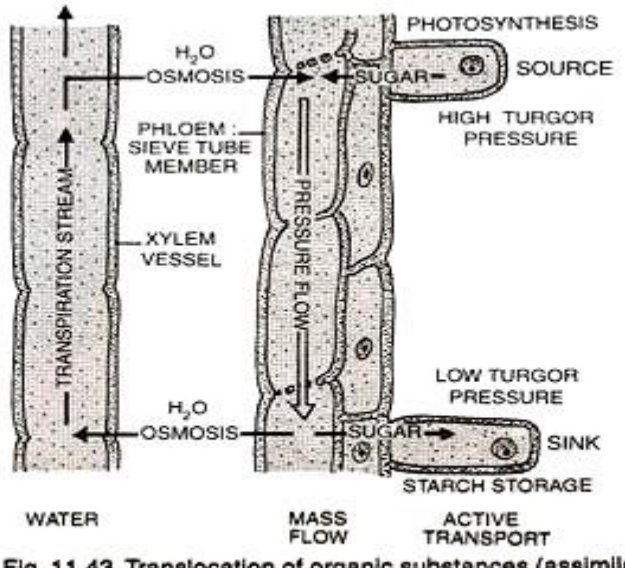


Fig. 11.43. Translocation of organic substances (assimilates) according to mass flow or pressure flow hypothesis.

It causes the flow of organic solution towards the area of low turgor pressure. A low turgor pressure is maintained in the sink region by converting soluble organic substances into insoluble form. Water passes back into xylem.

WATER POTENTIAL

INTRODUCTION OF WATER POTENTIAL

Most organisms are comprised of at least 70% or more water. Some plants, like a head of lettuce, are made up of nearly 95% water. When organisms go dormant, they loose most of their water.

For example, seeds and buds are typically less than 10% water, as are desiccated rotifers, nematodes and yeast cells. Earth is the water planet (that's why astronomers get so excited about finding water in space). Water is the limiting resource for crop productivity in most agricultural systems

LEARN MORE ABOUT WATER POTENTIAL • In general, water always moves down its water potential gradient from areas of higher water potential to areas of lower water potential.

- Water potential is typically measured as the amount of pressure needed to stop the movement of water.
- The unit used to express this pressure is the megapascal (MPa). The three factors that most commonly determine water potential are

WHAT IS WATER POTENTIAL?

Water potential is the potential energy of water relative to pure free water (e.g. deionized water) in reference conditions. It quantifies the tendency of water to move from one area to another due to osmosis, gravity, mechanical pressure, or matrix effects including surface tension.

Water potential is measured in units of pressure and is commonly represented by the Greek letter (Psi).

This concept has proved especially useful in understanding water movement within plants, animals, and soil.