# PINNACLE

## e-Proceedings of Departmental Seminars by the UG Students of Botany Honours

## DEPARTMENT OF BOTANY

Bethune College, Kolkata

# From Head Of The Department's Desk

*It gives me immense pleasure to see such a great seminar arranged online during these unprecedented times. The presenters this year were the 6th semester undergraduate students of Botany Honours. They presented their seminar on different aspects of Plant Physiology and Biochemistry. It was supervised and conducted by Dr. Tripti Roy and Dr. Debjani Sinha Roy, both Associate Professors of the Department of Botany. I would like to thank Dr. Tripti Roy for coming up with this concept and her efforts went a long way in making sure this program was a success.*

*The seminar has always been organized in person in the previous years but because of these pandemic we had to go online route.*

*This year twenty seven undergraduate students participated and presented their material on different areas of Plant Physiology and Biochemistry. Also this is first time the proceedings of the seminar have been published in the form an e book.*

*Taking all aspects into consideration the seminar was a great success and for that I reiterate my thanks to Dr. Tripti Roy and Dr. Debjani Sinha Roy .I would also like to thank other faculty members of our department for their support, encouragement and inspiration in organizing this event. I am deeply grateful to our students who presented their material online. Hopefully this serves as a rehearsal as they step on to bigger platforms during their masters programs. My blessings and love are always with them. My blessing is always with Arunika Roy for her efforts and technical support in publishing the seminar proceedings online.*

*To conclude,I hope our faculties would be able to conduct such student seminars every year which will definitely help them get accustomed to the system of delivering lectures at national and international forums.*

Dr. Ashok Kumar Das
Associate Professor & Head
P G Department of Botany
Bethune College

# From Editor's Desk

From Editor's Desk

*I have the pleasure to edit the proceedings of the online seminars held on and from 19th June 2021 to 6th July 2021 in Google Meet platform, delivered by the students of Botany Honours Semester VI in a situation when the world was in pandemic condition. Keeping aside the heartbreaking incidents in their surroundings, the participating students studied scientific topics from books and journals and focused the recent development of Plant Physiology and Biochemistry in seminars. They have followed the right lines to publish their findings. It was indeed nice experience to read the topics and hear their lectures. They have created history in the Post Graduate and Under Graduate Department of Botany, Bethune College for the first online publication of seminar proceedings, participated by the students.*

*I would like to express my thanks to Dr. Ashok Kumar Das, Head, Post Graduate and Under Graduate Department of Botany, Bethune College and Dr. Debjani Sinha Ray, Associate Professor of Botany, Bethune College for their continuous encouragement to the students and active presence in the series of seminars.*

*I also express my thanks and love to Arunika Paul, a student of B.Sc. Honours Semester VI, who assisted masterly for editing this proceeding as Assistant Editor.*

*I wish all-round success in the academic future of all my student participants.*

Dr. Tripti Roy
Associate Professor
PG Department of Botany
Bethune College

# From Assistant Editor's Desk

From Assistant Editor's Desk

*It was a great pleasure for me to become the Assistant Editor of the e- proceedings of the seminar series conducted by the Department of Botany, Bethune College in the academic session 2020-21. I am really grateful to the faculties of the department, particularly to Dr.Tripti Roy, Editor of this e-proceeding and Associate Professor, Bethune College for rendering me such a responsible job. I am thankful to all my friends who have extended full cooperation by providing their writings in due time. It was a great feeling for me to study all the papers of my beloved friends and this was the first time I acted as an Assistant Editor of e- proceedings. I enjoyed the job very much and I have learned a lot.*

Arunika Paul
Student of B.Sc Honours
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# MITOCHONDRIAL ELECTRON TRANSPORT SYSTEM IN PLANTS


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# Abstract

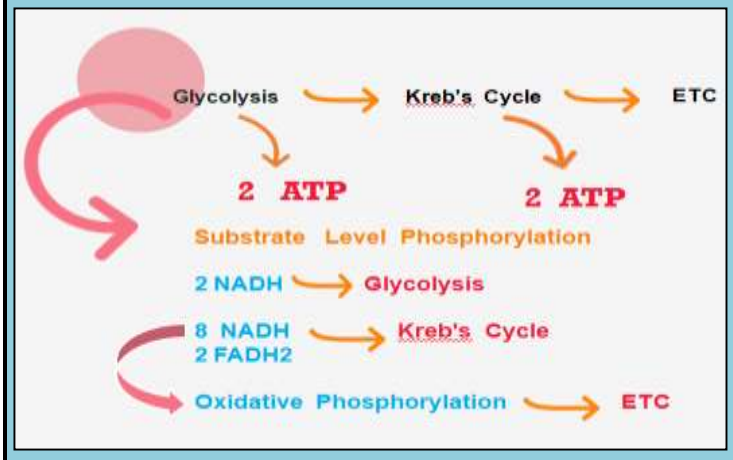
The electron transport chain is a cluster of proteins that transfer electrons through a membrane within mitochondria to form a gradient of protons that drives the creation of adenosine triphosphate (ATP).Three complexes are involved in this chain, namely, complex I, complex III, and complex IV. Some compounds like succinate, which have more positive redox potential than NAD+/NADH can transfer electrons via a different complex—complex II. Coenzyme Q, or simply Q, can travel within membrane while Cyt C is a soluble protein. Flavoproteins are components of complexes I and II and Fe-S is present in complexes I, II, and III. The Fe atom present in Fe-S complexes helps in electron transfer by shifting from Fe2+ to Fe3+ states. With the help of oxidation–reduction reactions a proton gradient is created which causes phosphorylation of ADP.The ETC passes electrons from NADH and FADH2 to protein complexes and mobile electron carriers. Coenzyme Q (CoQ) and cytochrome c (Cyt c) are mobile electron carriers in the ETC, and O2 is the final electron recipient. The malate and glycerol 3-P shuttles regenerate cytoplasmic NAD+ for glycolysis, and deliver reducing equivalents to the mitochondrial ETC. Inhibitors of oxidative phosphorylation arrest cellular respiration. Uncouplers dissociate oxidation from phosphorylation, and help to generate heat as animals adapt to the cold.

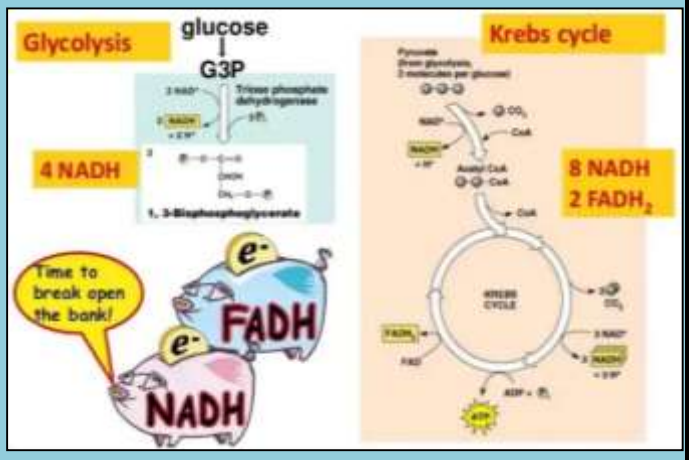
# Introduction

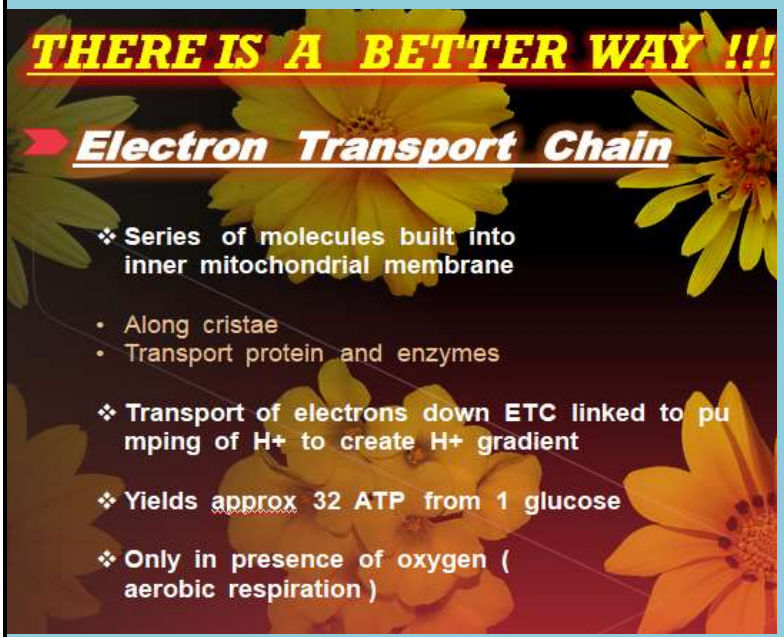
In oxidative phosphorylation, electrons are transferred along an electron transport chain consisting of a series of protein complexes embedded in the inner of the two mitochondrial membranes. This system transfers electrons from NADH (and related species)—produced by glycolysis,

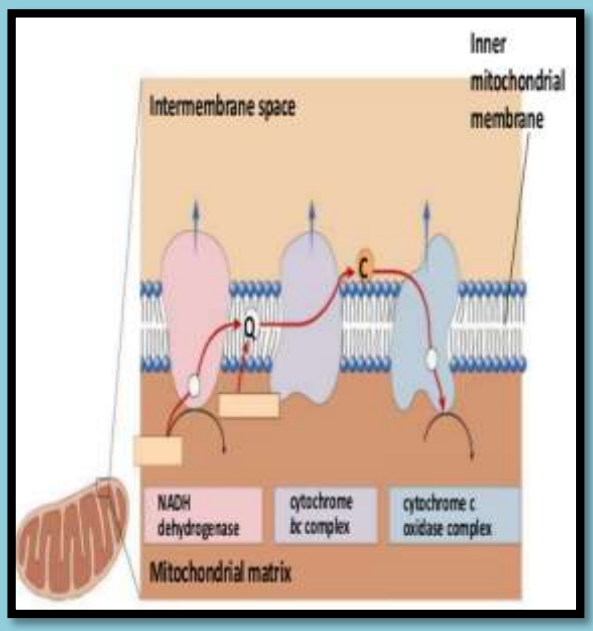
the oxidative pentose phosphate pathway, and the citric acid cycle—to oxygen. This electron transfer releases a large amount of free energy, much of which is conserved through the synthesis of ATP from ADP and Pi (inorganic phosphate), catalyzed by the enzyme ATP synthase. Collectively, the redox reactions of the electron transport chain and the synthesis of ATP are called oxidative phosphorylation.

## ATP ACCOUNTING SO FAR :-

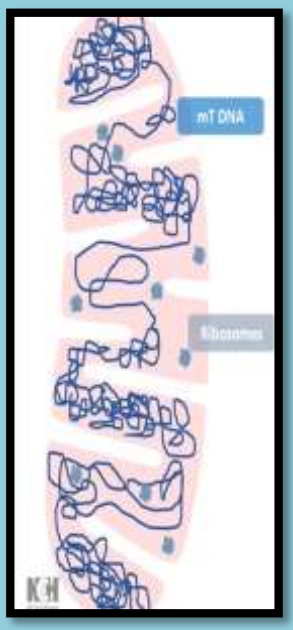


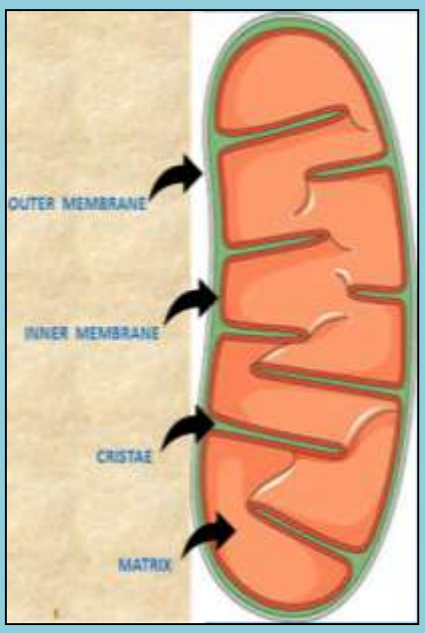



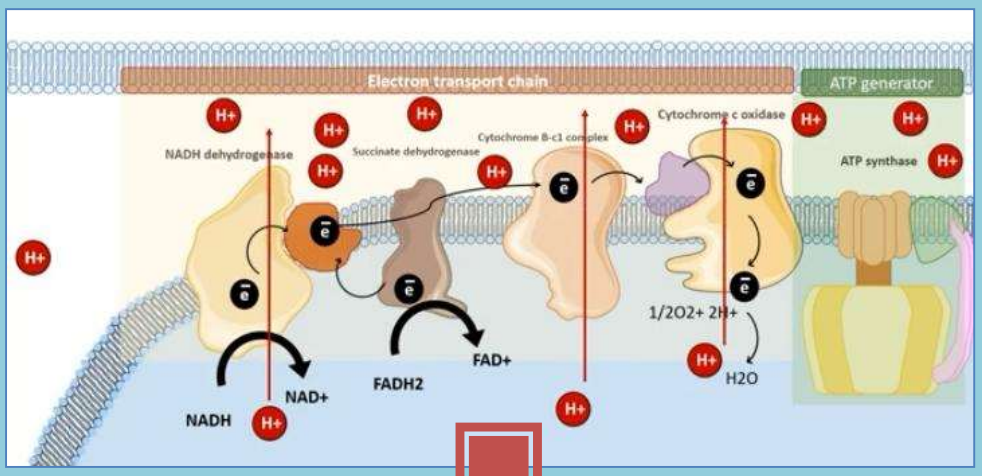



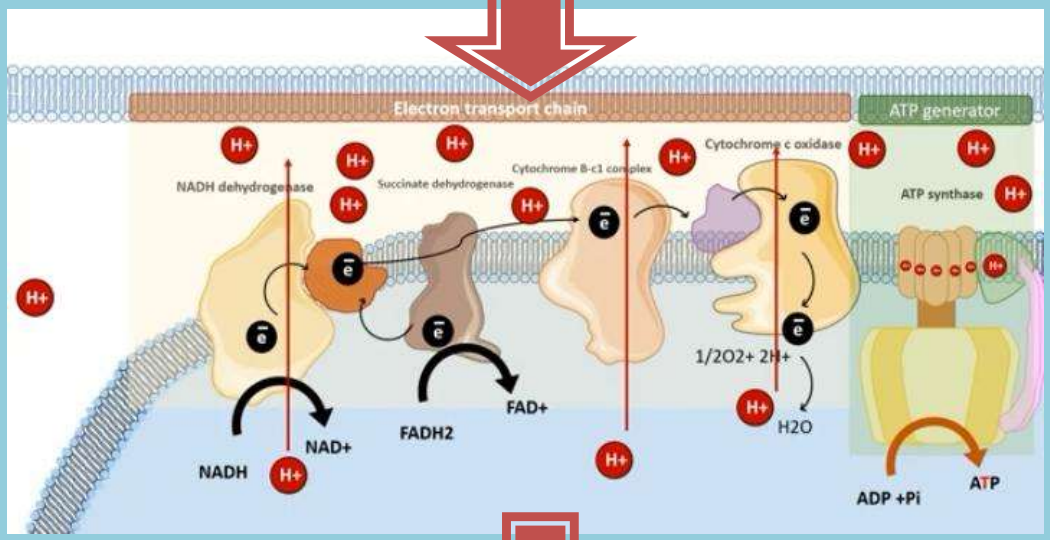



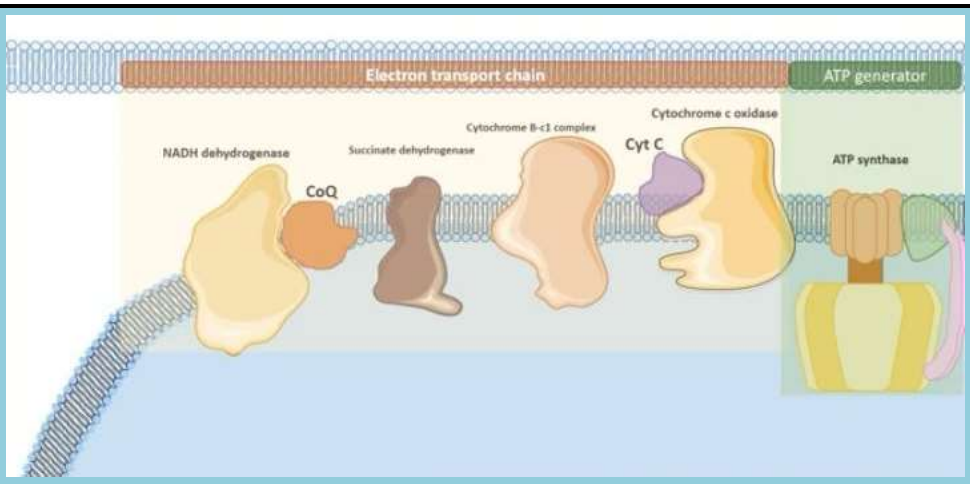
# MITOCHONDRIA






# COMPONENTS OF ETC

- **NAD & Flavoprotein** :H-carriers in celluiar respiration
- **Non heme metalloprotein (Fe-S- Protein)**:iron cycles between 3+ and 2+ states.
- **Ubiquinone or CoQ:** region serves as an anchor to inner mitochondrial membrane.
- **Cytochromes** : Electron-transfer proteins that contain a heme prosthetic group

## COMPOSITION OF ELECTRON TRANSPORT CHAIN

- Four large protein complexes.
- Complex I - NADH-Coenzyme Q reductase
- Complex II - Succinate-Coenzyme Q reductase
- Complex III - Cytochrome c reductase
- Complex IV - Cytochrome c oxidase
- Many of the components are proteins with prosthetic groups to move electrons.

# COMPLEX- I

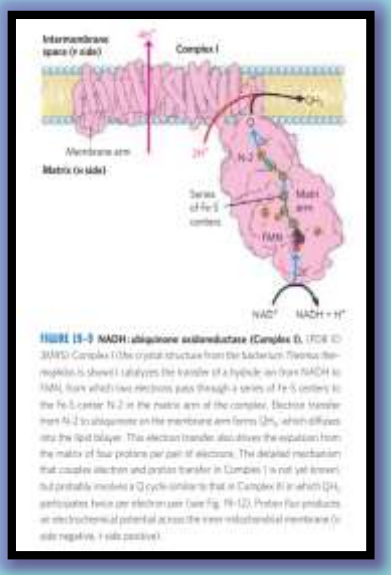
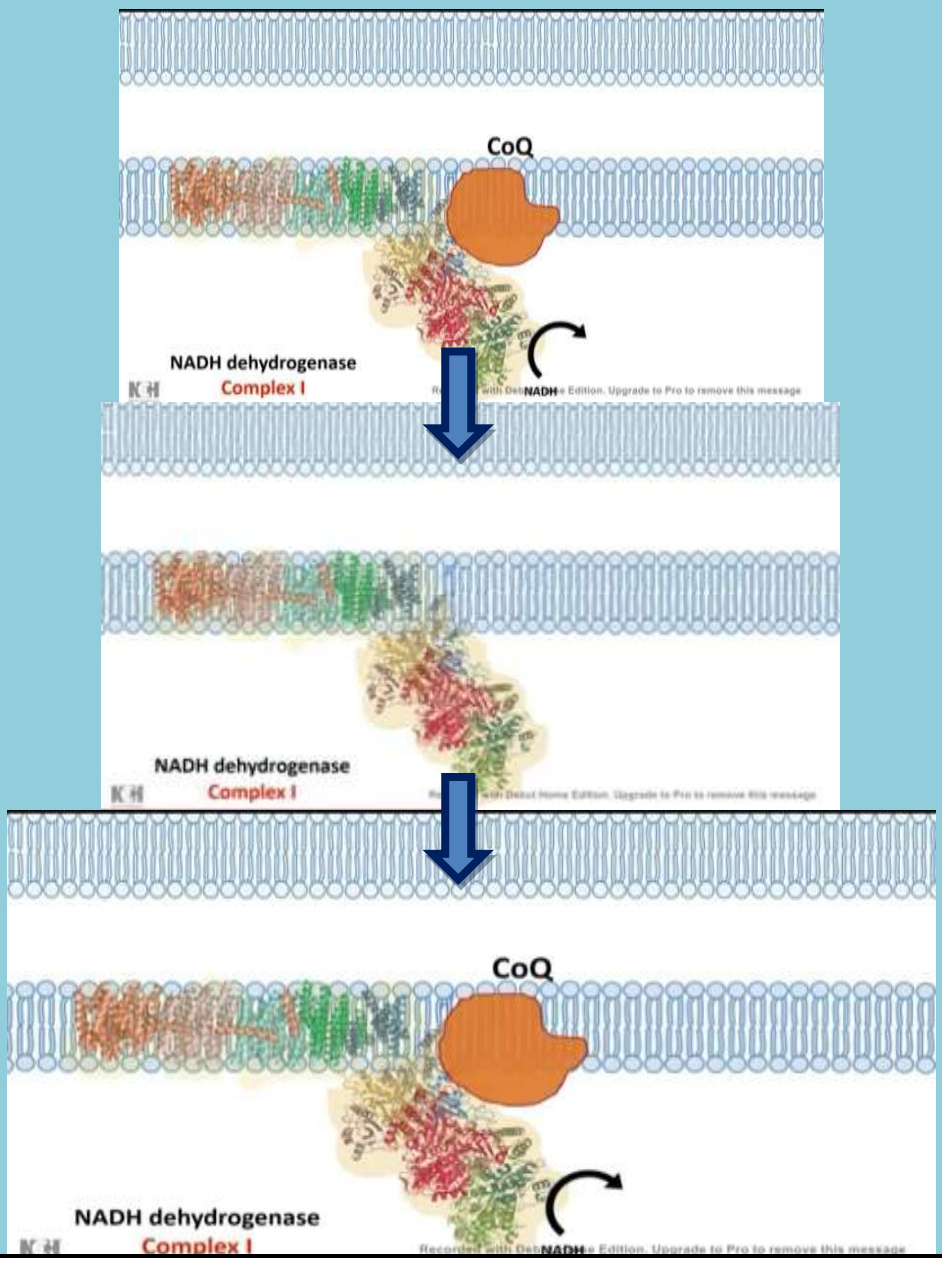



FIGURE 19-9 NADH: ubiquinone oxidoreductase (Complex I). (PDB ID 3M9S) Complex I (the crystal structure from the bacterium Thermus thermophilus is shown) catalyzes the transfer of a hydride ion (from NADH to FMN, from which two electrons pass through a series of Fe-S centers to the Fe-S center N-2 in the matrix arm of the complex. Electron transfer from N-2 to ubiquinone on the membrane arm forms $QH_2$, which diffuses into the lipid bilayer. This electron transfer also drives the expulsion from the matrix of four protons per pair of electrons. The detailed mechanism that couples electron and proton transfer in Complex I is not yet known, but probably involves a Q cycle similar to that in Complex III in which $QH_2$ participates twice per electron pair (see Fig. 19-12). Proton flux produces an electrochemical potential across the inner mitochondrial membrane (N side negative, P side positive).

- Complex I, also called NADH: ubiquinone oxidoreductase or NADH dehydrogenase, is a large enzyme composed of 42 different polypeptide chains, including an FMN-containing flavoprotein and at least six iron-sulfur centers.
- Complex I is L-shaped, with one arm of the L in the membrane and the other extending into the matrix.
- Complex I catalyzes two simultaneous and obligately coupled processes:
- (1) the exergonic transfer to ubiquinone of a hydride ion from NADH and a proton from the matrix, expressed by

$$NADH + H^+ + Q \longrightarrow NAD^+ + QH_2$$

- (2) the endergonic transfer of four protons from the matrix to the intermembrane space.
- Complex I is therefore a proton pump driven by the energy of electron transfer, and the reaction it catalyzes is vectorial: it moves protons in a specific direction from one location (the matrix, which becomes negatively charged with the departure of protons) to another (the intermembrane space, which becomes positively charged).



# COMPLEX- II

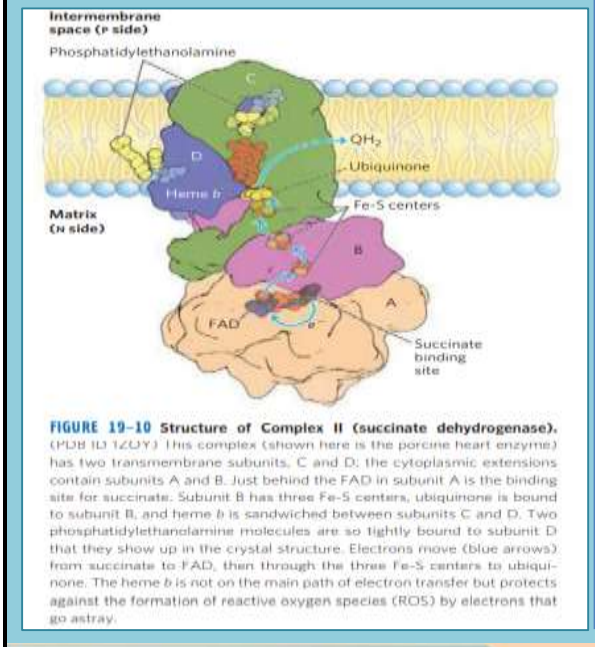
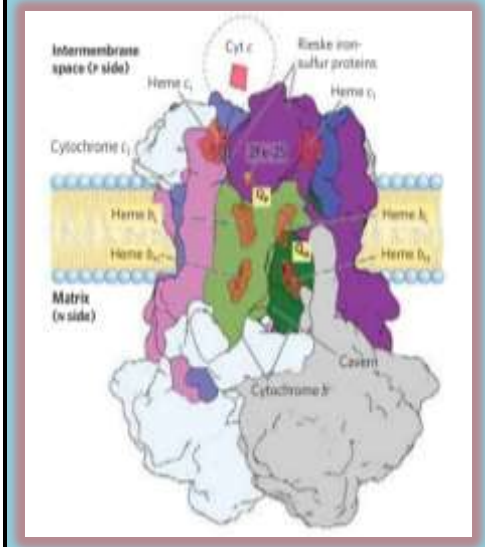


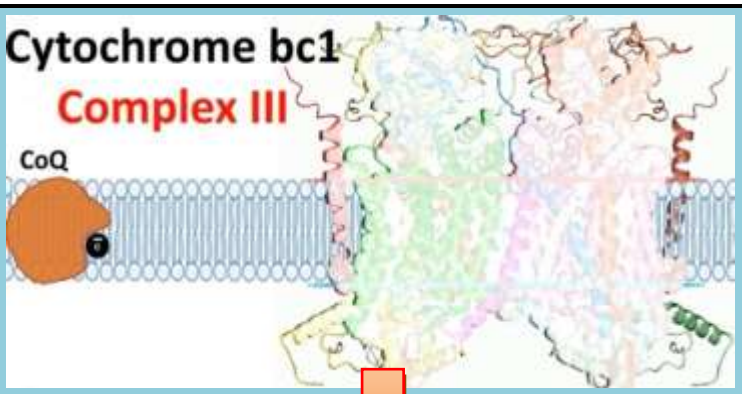

**FIGURE 19–10** Structure of Complex II (succinate dehydrogenase). (PDB ID 1ZOY) This complex (shown here is the porcine heart enzyme) has two transmembrane subunits, C and D; the cytoplasmic extensions contain subunits A and B. Just behind the FAD in subunit A is the binding site for succinate. Subunit B has three Fe-S centers; ubiquinone is bound to subunit B, and heme *b* is sandwiched between subunits C and D. Two phosphatidylethanolamine molecules are so tightly bound to subunit D that they show up in the crystal structure. Electrons move (blue arrows) from succinate to FAD, then through the three Fe-S centers to ubiquinone. The heme *b* is not on the main path of electron transfer but protects against the formation of reactive oxygen species (ROS) by electrons that go astray.

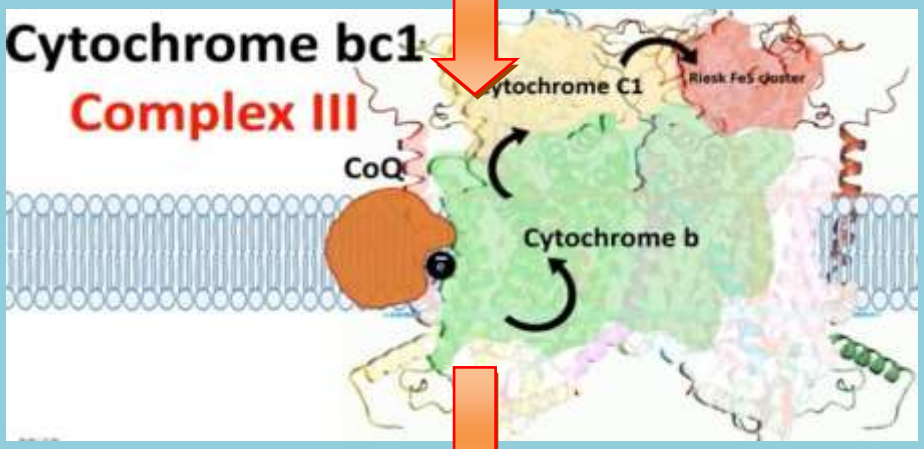
- Complex II: Succinate to Ubiquinone . We encountered Complex II as succinate dehydrogenase, the only membrane-bound enzyme in the citric acid cycle.
- Although smaller and simpler than Complex I, it contains five prosthetic groups of two types and four different protein subunits . Subunits C and D are integral membrane proteins, each with three transmembrane helices.
- They contain a heme group, heme b, and a binding site for ubiquinone, the final electron acceptor in the reaction catalyzed by Complex II. Subunits A and B extend into the matrix; they contain three 2Fe-2S centers, bound FAD, and a binding site for the substrate, succinate.
- The path of electron transfer from the succinate-binding site to FAD, then through the Fe-S centers to the Q-binding site, is more than 40 Å long, but none of the individual electron-transfer distances exceeds about 11 Å—a reasonable distance for rapid electron transfer.
- The heme b of Complex II is apparently not in the direct path of electron transfer; it may serve instead to reduce the frequency with which electrons "leak" out of the system, moving from succinate to molecular oxygen to produce the reactive oxygen species (ROS) hydrogen peroxide ($H_2O_2$) and the superoxide radical .

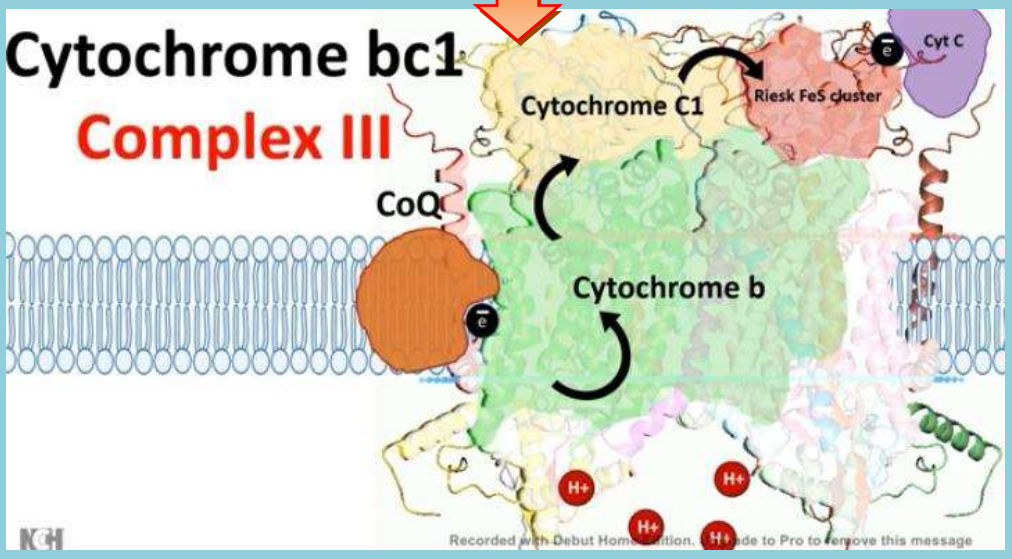
# COMPLEX- III



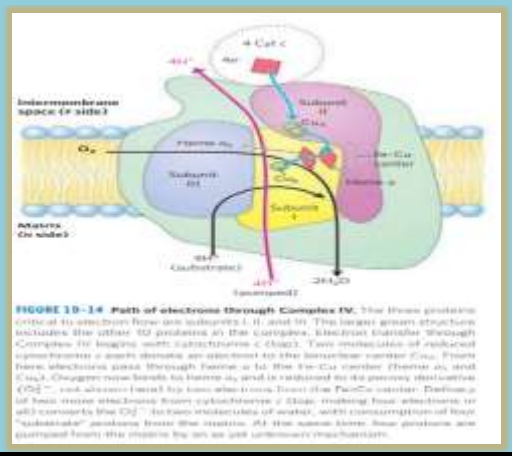

- Complex III: Ubiquinone to Cytochrome c. The next respiratory complex, Complex III, also called cytochrome bc1 complex or ubiquinone : cytochrome c oxidoreductase, couples the transfer of electrons from ubiquinol ($QH_2$) to cytochrome c with the vectorial transport of protons from the matrix to the intermembrane space
- The functional unit of Complex III is a dimer, with the two monomeric units of cytochrome b surrounding a "cavern" in the middle of the membrane, in which ubiquinone is free to move from the matrix side of the membrane (site QN on one monomer) to the intermembrane space (site QP of the other monomer) as it shuttles electrons and protons across the inner mitochondrial membrane
- Based on the structure of Complex III and detailed biochemical studies of the redox reactions, a reasonable model, the Q cycle, has been proposed for the passage of electrons and protons through the complex



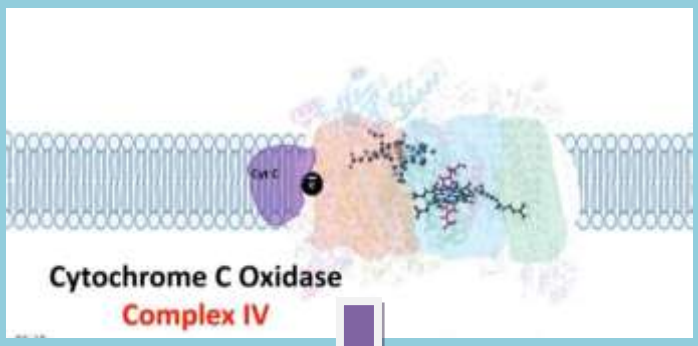



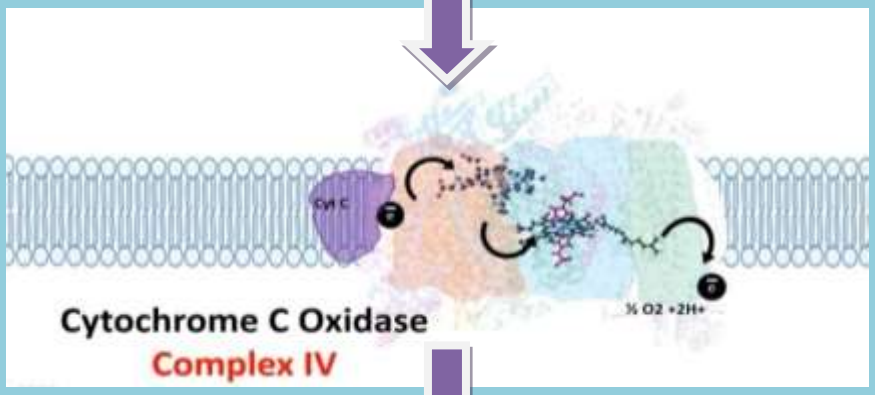

# COMPLEX- IV

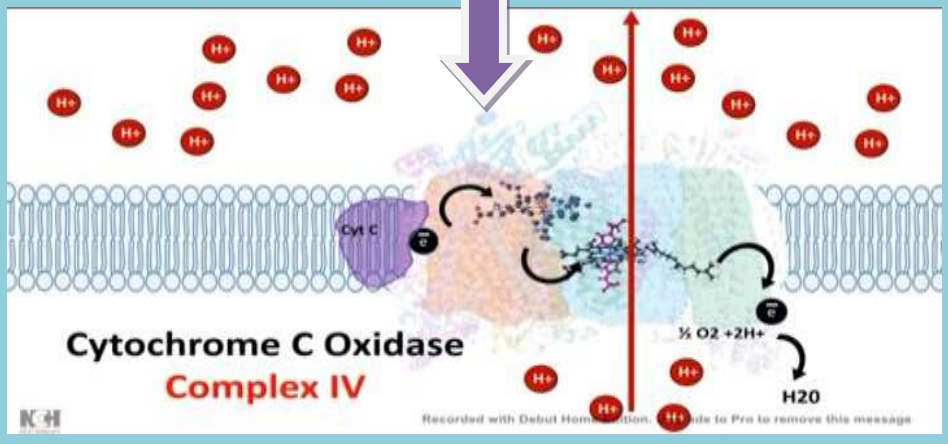


[15]

- Complex IV: Cytochrome c to O2 In the final step of the respiratory chain, Complex IV, also called cytochrome oxidase, carries electrons from cytochrome c to molecular oxygen, reducing it to H2O. Complex IV is a large enzyme (13 subunits; Mr 204,000) of the inner mitochondrial membrane.
- Mitochondrial subunit II contains two Cu ions complexed with the —SH groups of two Cys residues in a binuclear centerthat resembles the 2Fe-2S centers of iron-sulfur proteins. Subunit I contains two heme groups, designated a and a3 , and another copper ion (CuB). Heme a3 and CuB form a second binuclear center that accepts electrons from heme a and transfers them to O2 bound to heme a3 .
- Electron transfer through Complex IV is from cytochrome c to the CuA center, to heme a, to the heme a3–CuB center, and finally to O2 . For every four electrons passing through this complex, the enzyme consumes four "substrate" H from the matrix (N side) in converting O2 to 2H2O.

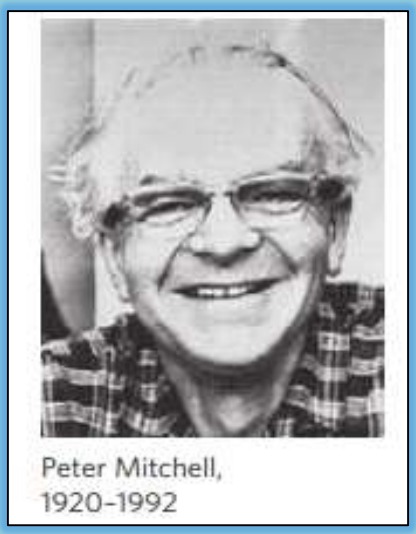


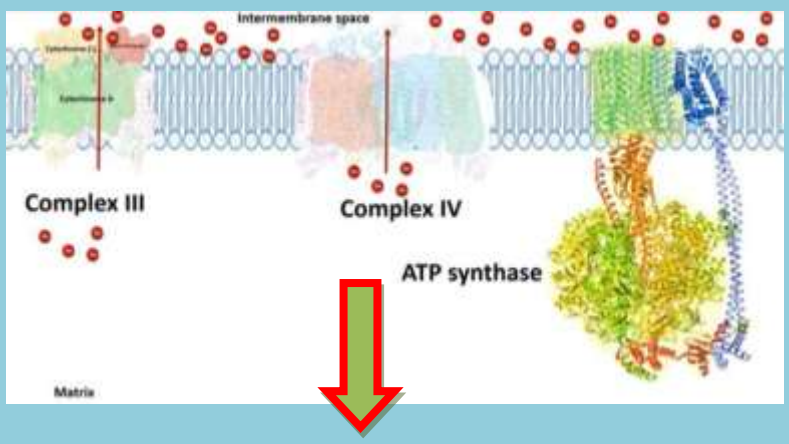



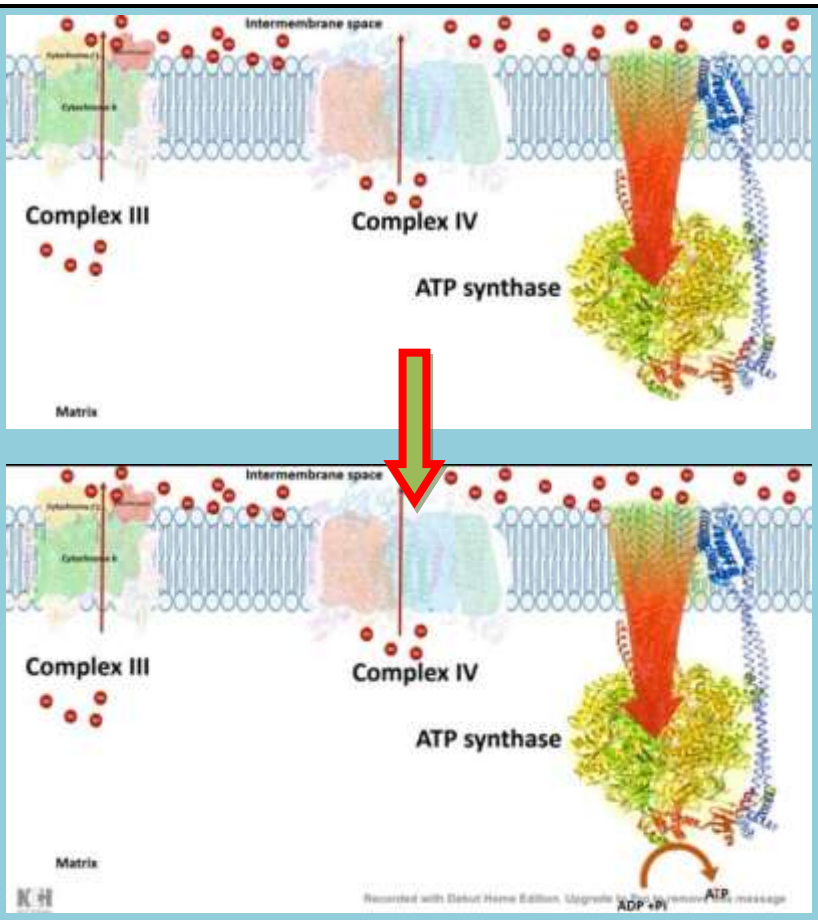



# OXIDATIVE PHOSPHORYLATION

- Oxidative phosphorylation is the name given to the synthesis of ATP (phosphorylation) that occurs when NADH and FADH2 are oxidized (hence oxidative) by electron transport through the respiratory chain.
- Unlike substrate level phosphorylation, it does not involve phosphorylated chemical intermediates. Rather, a very different mechanism was proposed by Peter Mitchell in 1961, the chemiosmotic hypothesis.
- It proposes that energy liberated by electron transport is used to create a proton gradient across the mitochondrial inner membrane and that it is this that is used to drive ATP synthesis.
- The actual synthesis of ATP is carried out by an enzyme called ATP synthase located in the inner mitochondrial membrane


Peter Mitchell,
1920–1992





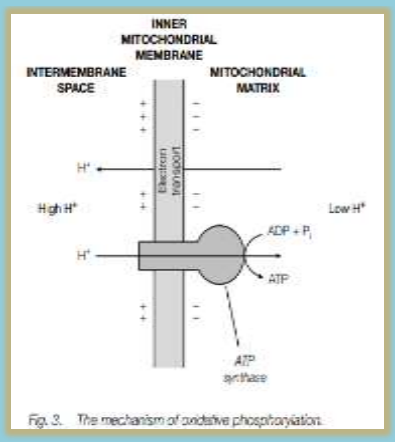



Fig. 3. The mechanism of oxidative phosphorylation.

# THE PROCESS IS AS FOLLOWS :-

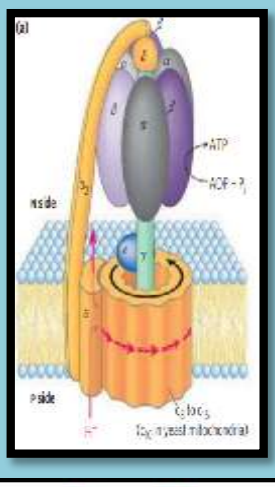
- Electron transport down the respiratory chain from NADH oxidation causes H+ ions to be pumped out of the mitochondrial matrix across the inner mitochondrial membrane into the intermembrane space by the three H + pumps; Complex I, III and IV . [Because FADH2 is reoxidized via ubiquinone, its oxidation causes H + ions to be pumped out only by Complex III and IV and so the amount of ATP made from FADH2 is less than from NADH.]

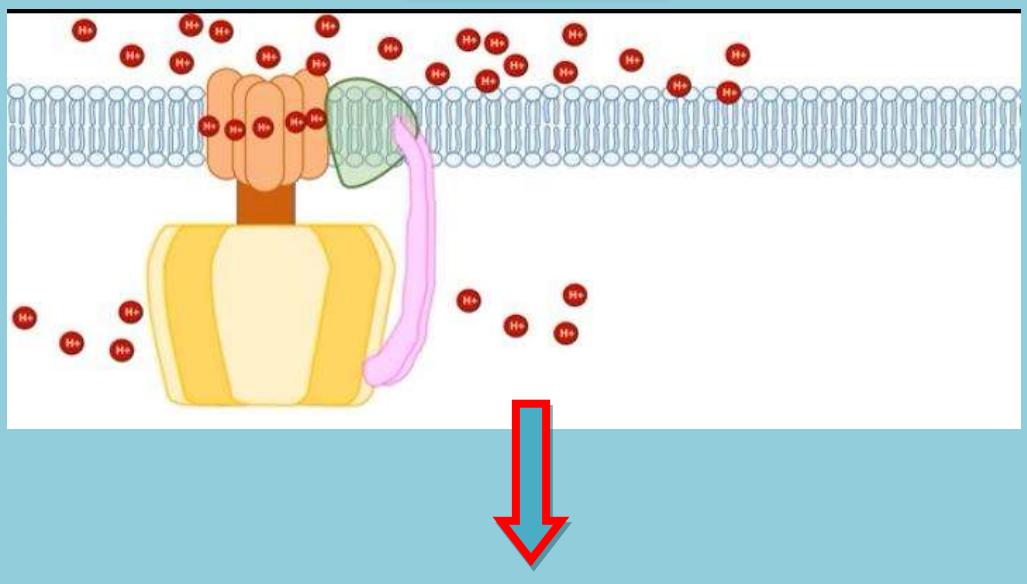
- The free energy change in transporting an electrically charged ion across a membrane is related both to its electrical charge and the concentration of the species.
- The pumping out of the H + ions generates a higher concentration of H + ions in the intermembrane space and an electrical potential, with the side of the inner mitochondrial membrane facing the intermembrane space being positive . Thus, overall, an electrochemical proton gradient is formed.
- The protons flow back into the mitochondrial matrix through the ATP synthase and this drives ATP synthesis. The ATP synthase is driven by proton-motive force, which is the sum of the pH gradient (i.e. the chemical gradient of H + ions) and the membrane potential (i.e. the electrical charge potential across the inner mitochondrial membrane).
- There is some debate over the exact stoichiometry of ATP production; in past years it was believed that 3 ATPs were generated per NADH and 2 ATPs per FADH2 but some recent measurements have indicated that the numbers of ATP molecules generated may be 2.5 and 1.5, respectively .

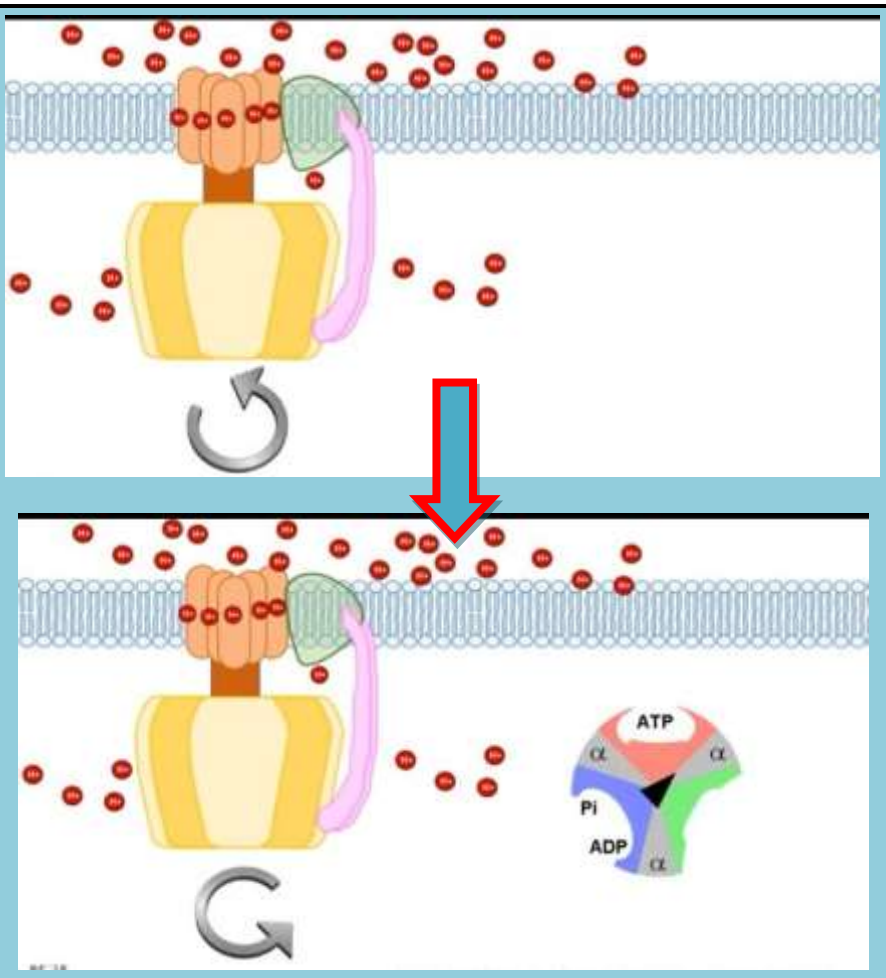
## ATP SYNTHASE AS A ROTATORY ENGINE

- The ATP synthase can be seen as spherical projections from the inner membrane . If mitochondria are subjected to sonic disruption, submitochondrial vesicles are formed in which the spheres of the ATP synthase point outward .
- In 1960, Racker showed that the spheres can be removed and that the isolated spheres hydrolyze ATP, that is, the spheres have ATPase activity (called F1 ATPase); . The stripped submitochondrial vesicles, devoid of the F1 ATPase, can still transport electrons along the electron transport chain but cannot synthesize ATP. These stripped submitochondrial vesicles contain the other major part of the ATP synthase, called F0 (coupling factor 0) which spans the inner mitochondrial membrane .

- Since it is composed of these two major component parts, ATP synthase is also known as F0F1 ATPase. The complete complex harnesses the energy released by electron transport to drive ATP synthesis whereas alone, without coupling to electron transport, the F1 component hydrolyzes ATP.
- The F1 ATPase consists of five types of polypeptides in the following ratio: α3, β3, γ, δ, ε. The six α and β subunits are arranged alternately in a ring, with a central stalk formed by the γ and ε subunits .
- F0 consists of a ring of 10 to 14 c subunits sitting in the inner mitochondrial membrane. This contacts a single a subunit that links to two b subunits and the single δ subunit to form a long column that connects to the head of the F1 ATPase .
- This overall structure, the F0F1 ATPase, is a remarkable molecular motor.



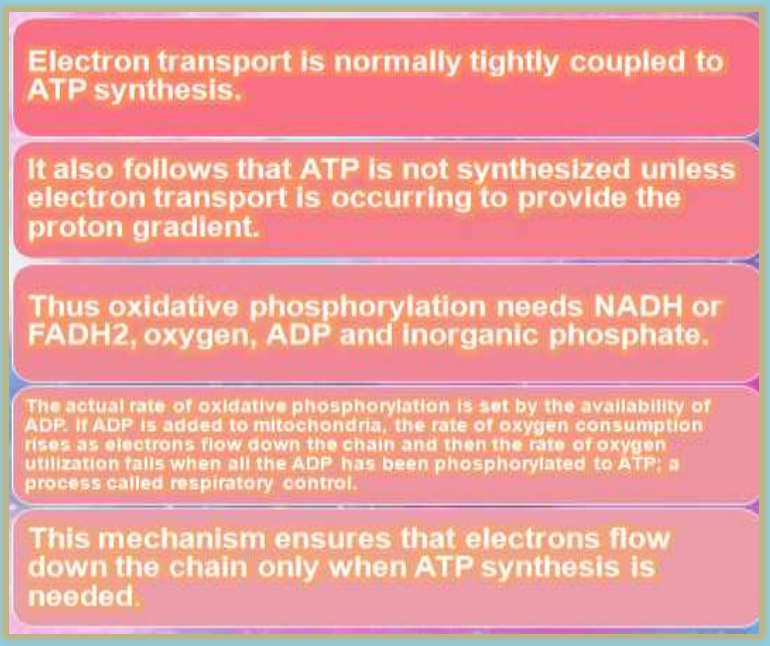



# ELECTRON TRANSPORT INHIBITORS

Several inhibitors of specific electron carriers are known and were used in the original studies to determine the order of the components in the respiratory chain. For example:

- rotenone and amytal inhibit electron transport at NADH-Q reductase and so prevent NADH oxidation but the oxidation of FADH2 can still occur since this feeds electrons into the chain at CoQ (i.e. past the point of inhibition).
- antimycin A inhibits electron transport at the Q-cytochrome c reductase complex.
- cyanide(CN–), azide (N3 –) and carbon monoxide (CO) all inhibit cytochrome c oxidase.

# COUPLING AND RESPIRATORY CONTROL




## UNCOUPLERS

- Some chemicals, such as 2,4-dinitrophenol (DNP), act as uncoupling agents, that is, when added to cells, they stop ATP synthesis but electron transport still continues and so oxygen is still consumed.
- The reason is that DNP and other uncoupling agents are lipid-soluble small molecules that can bind H+ ions and transport them across membranes (i.e. they are H+ ionophores).
- Electron transport occurs and pumps out H+ ions across the inner mitochondrial membrane but DNP in the same membrane carries the H+ ions back into the mitochondrion, preventing formation of a proton gradient.
- Since no proton gradient forms, no ATP can be made by oxidative phosphorylation. Rather the energy derived from electron transport is released as heat. The production of heat by uncoupling is called nonshivering thermogenesis.
- It is important in certain biological situations. For example, uncoupling occurs naturally in brown adipose tissue. This tissue is rich

in mitochondria, the inner mitochondrial membranes of which contain a protein called thermogenin (or uncoupling protein)

**TABLE 19-5** ATP Yield from Complete Oxidation of Glucose

| Process | Direct product | Final ATP |
|---|---|---|
| Glycolysis | 2 NADH (cytosolic) | 3 or 5* |
| | 2 ATP | 2 |
| Pyruvate oxidation (two per glucose) | 2 NADH (mitochondrial matrix) | 5 |
| Acetyl-CoA oxidation in citric acid cycle (two per glucose) | 6 NADH (mitochondrial matrix) | 15 |
| | 2 $FADH_2$ | 3 |
| | 2 ATP or 2 GTP | 2 |
| Total yield per glucose | | 30 or 32 |

# Conclusion

Each of the three cellular respiration phases incorporates important cell processes, but the ETC produces by far the most ATP. Since energy production is one of the key functions of cell respiration, ATP is the most important phase from that point of view.

Where the ETC produces up to **34 molecules of ATP** from the products of one glucose molecule, the citric acid cycle produces two, and glycolysis produces four ATP molecules but uses up two of them.

The other key function of the ETC is to produce **NAD** and **FAD** from NADH and FADH in the first two chemical complexes. The products of the reactions in ETC complex I and complex II are the NAD and FAD molecules that are required in the citric acid cycle.

As a result, the citric acid cycle is dependent on the ETC. Since the ETC can only take place in the presence of oxygen, which acts as the

final electron acceptor, the cell respiration cycle can only operate fully when the organism takes in oxygen.

# Acknowledgement

I would like to thank my respected teachers, Dr.Debjani Sinha Ray and Dr.Tripti Roy for giving me this opportunity of making this presentation. It was a great learning experience. I have gained a lot of knowledge during the course of making this presentation. And lastly I would like to thank everyone who helped me in any way they could for making this presentation a success.

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# Paper-II

# Nitrate Assimilation with special Emphasis on Ammonium Assimilation Through GSGOGAT System

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# Abstract

Nitrogen assimilationin the soil generally occurs through two form like **Nitrate ($NO_3^-$)** & **Ammonium ($NH_4^+$)** by sequestering the exceptionally stable triple covalent bond between two nitrogen atoms.In this topic I want to bring the topic **NITRATE ASSIMILATION WITH SPECIAL REFERENCE TO GS-GOGAT SYSTEM** in light.

**Nitrate assimilation** is generally done through the key role of 2 enzymes **Nitrate reductase & Nitrite reductase** by converting **Nitrate into Nitrite & Nitrite into Ammonium** in **Roots** & in some plants **Shoots too** (**Xanthium strumarium**)

The **Ammonium** is then converted into **Amino acid** which is generated through **Nitrate assimilation or photorespiration** .**Ammonia** assimilated through 2 pathway

**1. Primary pathway (**incorporating **GS- GOGAT** system)
**2. Alternative pathway** (incorporating **GDH, TRANSAMINASE/AMINOTRANSFERASE )**

**Inspite of fixing ammonia by an alternative pathway ammonia can be mainly assimilated via primary pathway or GS-GOGAT System.**
**The derived Amino acid biologically synthesisized and fulfilled the requirement of amino acid inside te plant body.**

But we have to remember that unassimilated Nitrate or Ammonium may be dangerous for plant body itself.

In this topic I have covered these things to give a clear idea about nitrate assimilation with special reference to Ammonium assimilation through GSGOGAT system.

# Introduction

Nitrogen passes through several forms in a biogeochemical cycle. The atmosphere contains vast quantities (about 78% by volume) of molecular nitrogen ($N_2$). For the most part, this large reservoir of nitrogen is not directly available to living organisms. Acquisition of nitrogen from the atmosphere requires the breaking of an exceptionally

stable triple covalent bond between two nitrogen atoms (N≡N) to produce ammonia ($NH_3$) or nitrate ($NO_3^-$). These reactions, known as nitrogen fixation, occur through both industrial and natural processes.

- In nitrate ($NO_3^-$) assimilation, the nitrogen in $NO_3^-$ is converted to a higher-energy (more reduced) form in nitrite ($NO_2^-$), then to a yet higher-energy (even more reduced) form in ammonium ($NH_4^+$), and finally into the amide nitrogen of the amino acid glutamine.

This process consumes protonated to form the ammonium ion ($NH_4^+$). The process of biological nitrogen fixation, together with the subsequent assimilation of $NH_3$ into an amino acid, consumes the equivalent of about 16 ATPs per amide nitrogen $N_2$ the equivalent of 12 ATPs per amide nitrogen.

- Plants such as legumes form symbiotic relationships with nitrogen fixing bacteria to convert molecular nitrogen ($N_2$) into ammonia ($NH_3$). Ammonia ($NH_3$) is the first stable product of natural fixation; at physiological pH, however, ammonia is protonated to form the ammonium ion ($NH_4^+$). The process of biological nitrogen fixation, together with the subsequent assimilation of $NH_3$ into an amino acid, consumes the equivalent of about 16 ATPs per amide nitrogen.

## THE NITROGEN FIXED IN THE ENVIRONMENT THROUGH FOLLOWING THREE PROCESSES:

**1.Lightening**
**2.Photochemical reaction**
**3.Biological nitrogen fixation.**

## Among the three processes nitrogen is fixed to ammonia in biological nitrogen fixation

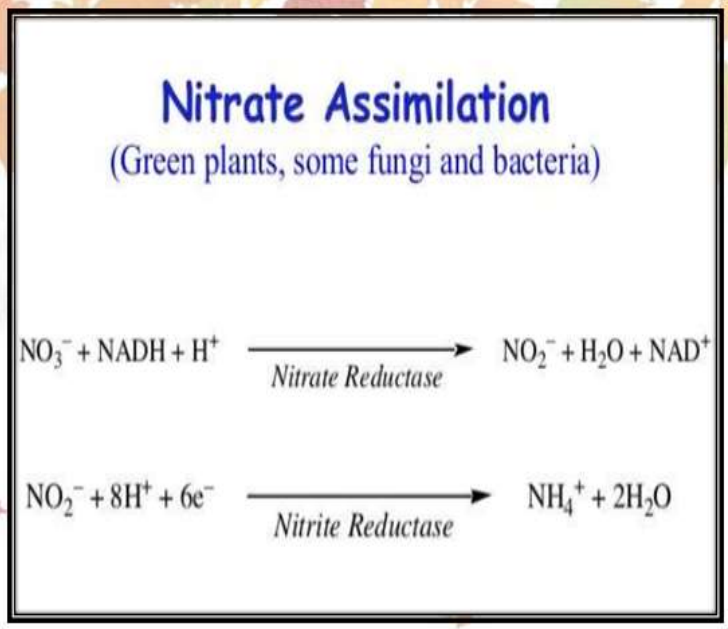
- **Biological nitrogen fixation. The remaining 90% results from biological nitrogen fixation, in which bacteria or cyanobacteria (blue green algae) fix $N_2$ into ammonia ($NH_3$). This ammonia dissolves in water to form ammonium**

$$(NH_4+): NH_3 + H_2O \rightarrow NH_4^+ + OH^-$$

# NITRATE ASSIMILATION

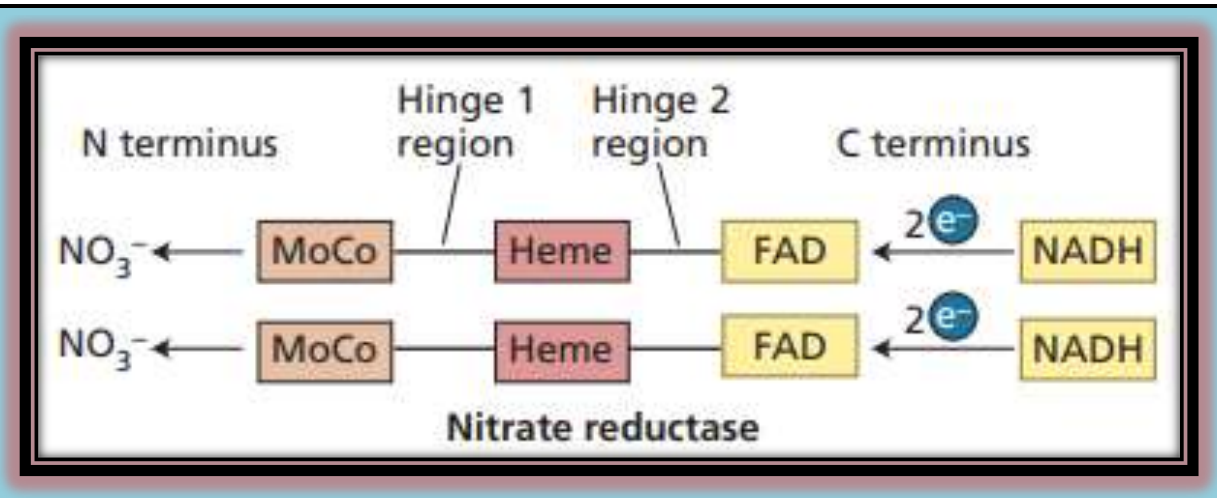
Plants eventually assimilate most of this **Nitrate** into organic nitrogen compounds.

The first step of this process is the conversion of **Nitrate($NO_3^-$) to Nitrite($NO_2^-$) with the help of Nitrate reductase.**
**The second step is the conversion of nitrate to nitrite ion.**
**In this process following enzyme plays the key role.**




# NITRATE REDUCTASE

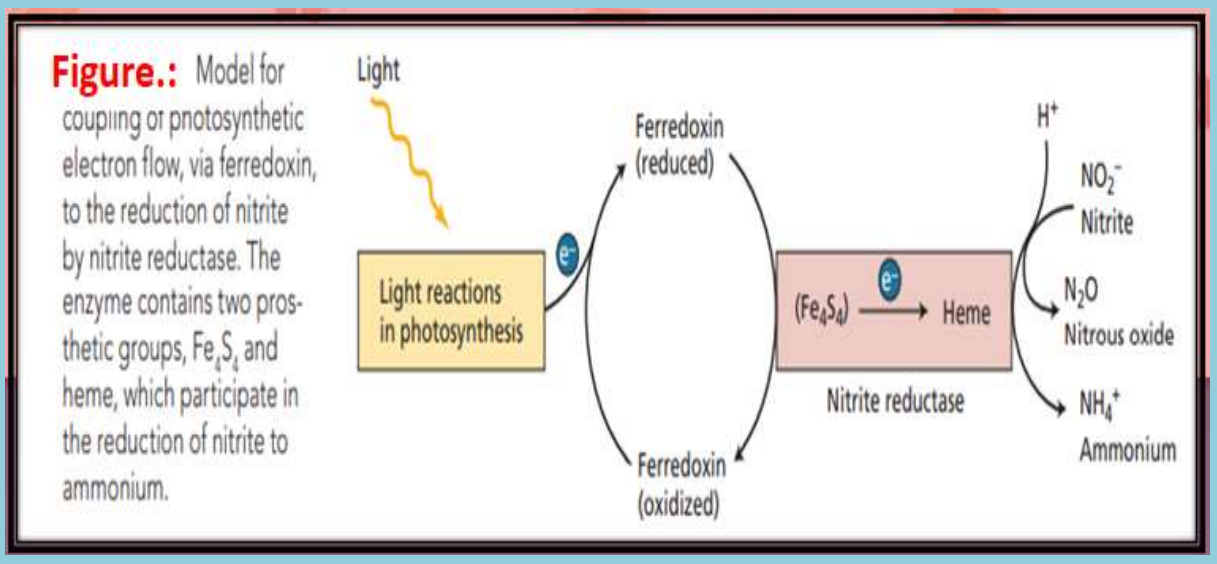
In the cytosol, a reduction reaction that involves the transfer of two electrons. The enzyme **Nitrate Reductase** catalyzes this reaction.




## NITRITE REDUCTASE:
## THIS ENZYME CONVERTSNITRITE($NO_2^-$) TOAMMONIUM($NH_4^+$).

A highly reactive, potentially toxic ion. Plant cells immediately transport the nitrite generated by nitrate reduction from the cytosol into chloroplasts in leaves and plastids in roots. In these organelles, the enzyme **Nitrite reductase reduces Nitrite to Ammonium**, a reaction that involves the transfer of six electrons, according to the following overall reaction:

$$NO_2^- + 6\,Fdred + 8\,H^+ \rightarrow NH_4^+ + 6\,Fdox + 2H_2O$$



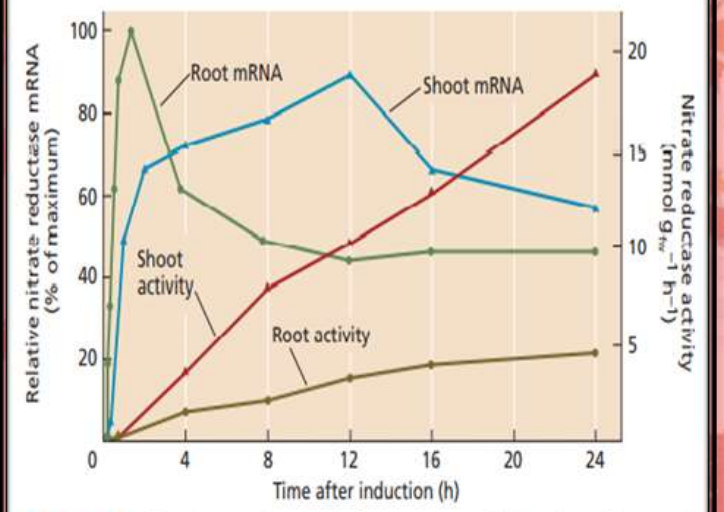



**Figure.:** Stimulation of nitrate reductase activity follows the induction of nitrate reductase mRNA in shoots and roots of barley; $g_{fw}$, grams fresh weight. (After Kleinhofs et al. 1989.)

## PLACE:

Mainly nitrate assimilated in the roots, here root hair absorbed the nitrate ion from soil solution. But some times it is stored in shoot also.

In plants such as cocklebur (*Xanthium strumarium*), nitrate metabolism is restricted to the shoot; in otherplants, such as white lupine (*Lupinus albus*), most nitrate is metabolized in the roots. Generally, species native to temperate regions rely more heavily on nitrate assimilation by the roots than do species of tropical or subtropical origins.

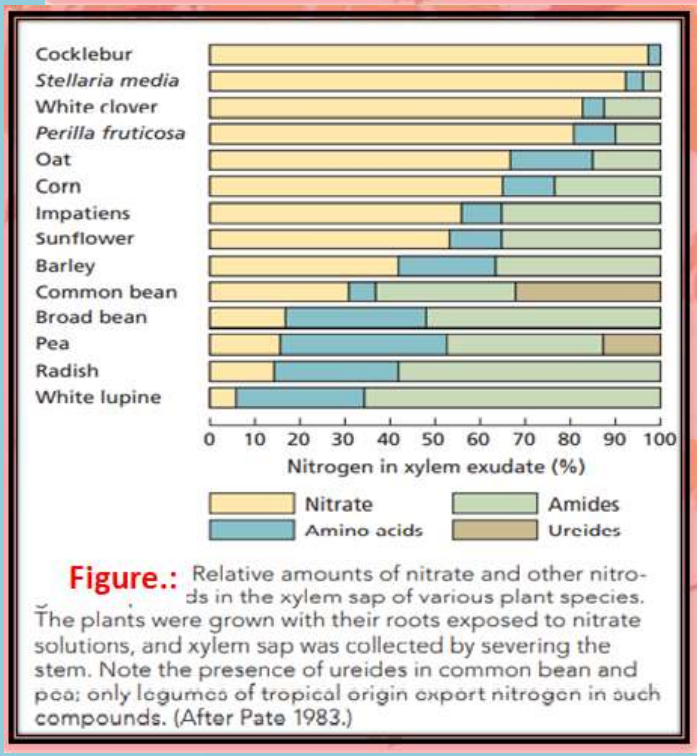



**Figure.:** Relative amounts of nitrate and other nitrogenous compounds in the xylem sap of various plant species. The plants were grown with their roots exposed to nitrate solutions, and xylem sap was collected by severing the stem. Note the presence of ureides in common bean and pea; only legumes of tropical origin export nitrogen in such compounds. (After Pate 1983.)

Nitrate reductase activity varies among species to species depending on concencentration of nitrate ion and reduced nitrogen in xylem sap.

## Ammonia Assimilation

- Conversion of ammonia generated from nitrate assimilation or photorespiration into amino acid.
- 2 pathways – i. Primary Pathway
  ii. Alternative Pathway

# AMMONIA CAN BE ASSIMILATED THROUGH TWO PATHWAYS MENTIONED BELOW:

## Primary Pathway

2 enzymes involved :

a) Glutamine synthetase (GS) – Available in two forms- in cytosol , and plastid/shoot chloroplast

a) Glutamate synthase (also known as GOGAT)

GOGAT – Glutamine 2-oxo-glutarate aminotransferase

## Alternative Pathway

- The enzyme involved is Glutamate Dehydrogenase (GDH), hence known as GDH pathway.
- Catalyzes synthesis and deamination of glutamate.
- Reversible

(1) (2)

# PRIMARY PATHWAY

## GLUTAMINE SYNTHETASE (GS):
## IT COMBINES AMMONIUM WITH GLUTAMATE TO FORM GLUTAMINE

**Glutamate + $NH_4$ + + ATP → glutamine + ADP + Pi .**

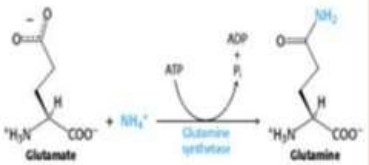
This reaction requires the hydrolysis of one ATP and involves a divalent cation such as $Mg_2^+$, $Mn_2^+$, or $CO_2^+$ as a cofactor.

Light and carbohydrate levels alter the expression of the plastid forms of the enzyme, but they have little effect on the cytosolic forms.

### Glutamine synthetase action

**Cytosolic form –**

- Expressed in germinating seeds or in vascular bundle of roots and shoots.
- Produce glutamine for intracellular nitrogen transport.




**Root plastid/Shoot chloroplast form –**

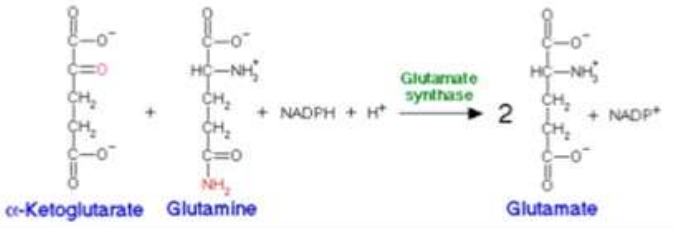
- In roots, it produces amide nitrogen for local consumption.
- In shoots, it reassimilates photorespiratory $NH_4^+$

# GLUTAMATE SYNTHASE:

Elevated plastid levels of **glutamine**(GS) stimulate the activity of **Glutamate synthase (also known as glutamine:2- oxoglutarate aminotransferase, or GOGAT)**

## Glutamate synthase action

- Transfers amide group of glutamine to 2-oxoglutarate, and yields two glutamate molecules.




## Types of GOGAT

- **NADH-GOGAT**
  - accepts electrons from NADH.
  - located in plastids of non-photosynthetic tissue like roots or VB of developing leaves.

Glutamine + 2-oxoglutarate + NADH + $H^+$ ⟶

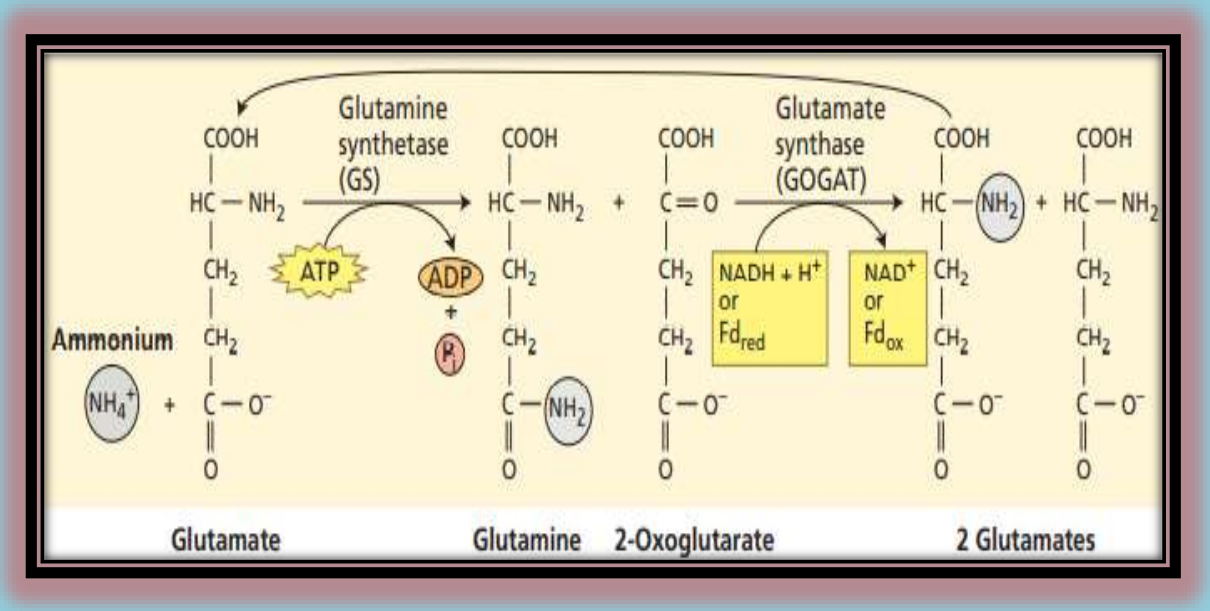
2 Glutamate + $NAD^+$

- **Fd-GOGAT**
  - accepts electron from ferrodoxin.
  - located in chloroplast and serves in photorespiratory nitrogen metabolism.

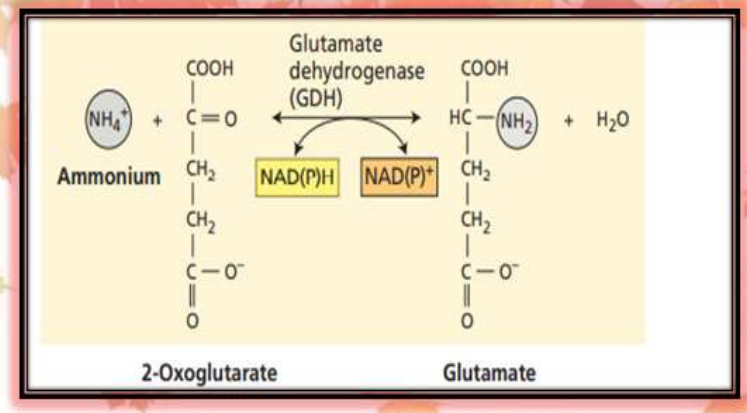
Glutamine + 2-oxoglutarate + $Fd_{red}$ ⟶ 2 Glutamate + $Fd_{ox}$

**In roots, NADH-GOGAT is involved in the assimilation of $NH4^+$ absorbed from the rhizosphere. In vascular bundles of developing leaves, NADH-GOGAT assimilates glutamine translocated from roots or senescing leaves.**

**Fd-GOGAT in the roots presumably functions to incorporate the glutamine generated during nitrate assimilation.**

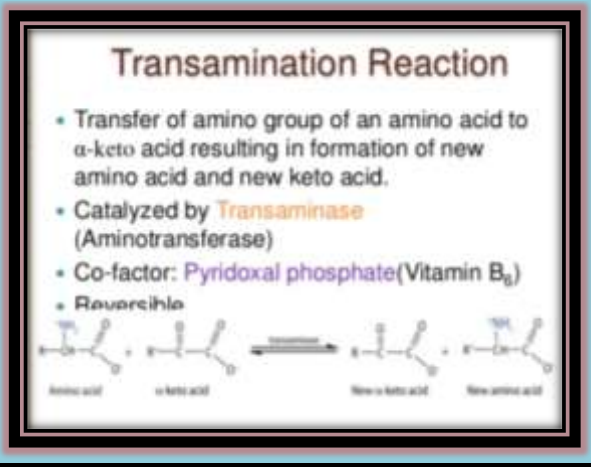



# ALTERNATIVE PATHWAY



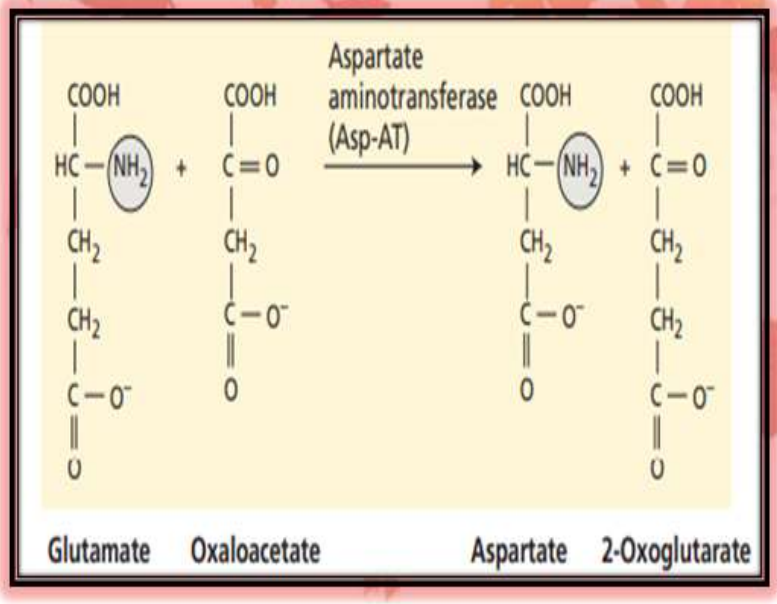

$$\text{2-Oxoglutarate} + NH_4^+ + \text{NAD(P)H} \leftrightarrow \text{glutamate} + H_2O + \text{NAD(P)}^+$$

An NADH-dependent form of GDH is found in mitochondria, and a NADPH dependent form of GDH is localized in the chloroplasts of photosynthetic organs. Although both forms are relatively abundant, **they cannot substitute for the GS–GOGAT pathway for assimilation of ammonium**, and their primary function is in deaminating glutamate during the reallocation of nitrogen.



Once assimilated into glutamine and glutamate, nitrogen is incorporated into other amino acids via transamination reactions. The enzymes that catalyze these reactions are known as Aminotransferases( transaminase). An example is Aspartate aminotransferase (Asp-AT), which catalyzes the following reaction:

**Glutamate + oxaloacetate → 2-oxoglutarate + aspartate**



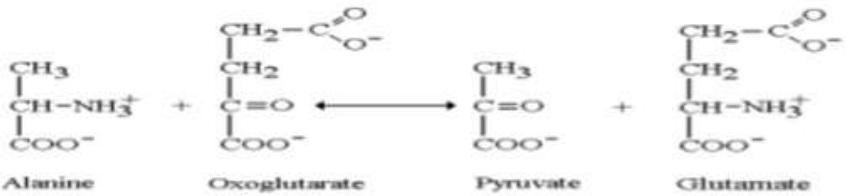

Aminotransferases are found in the cytoplasm, chloroplasts, mitochondria, glyoxysomes, and peroxisomes.
The aminotransferases in chloroplasts may have a significant role in amino acid biosynthesis, because plant leaves or isolated chloroplasts exposed to radioactively labeled carbon dioxide rapidly incorporate the label into glutamate, aspartate, alanine, serine, and glycine.

## SOME OTHER EXAMPLE OF TRANSAMINASE ARE AS FOLLOWS:

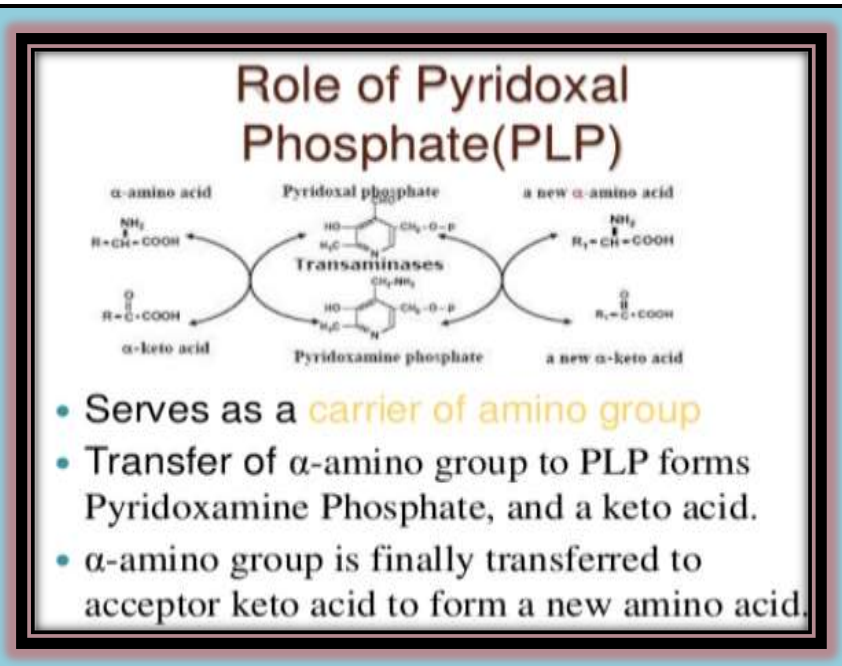
Example:

- Catalyzed by Aspartate Transaminase


- Catalyzed by Alanine Transaminase



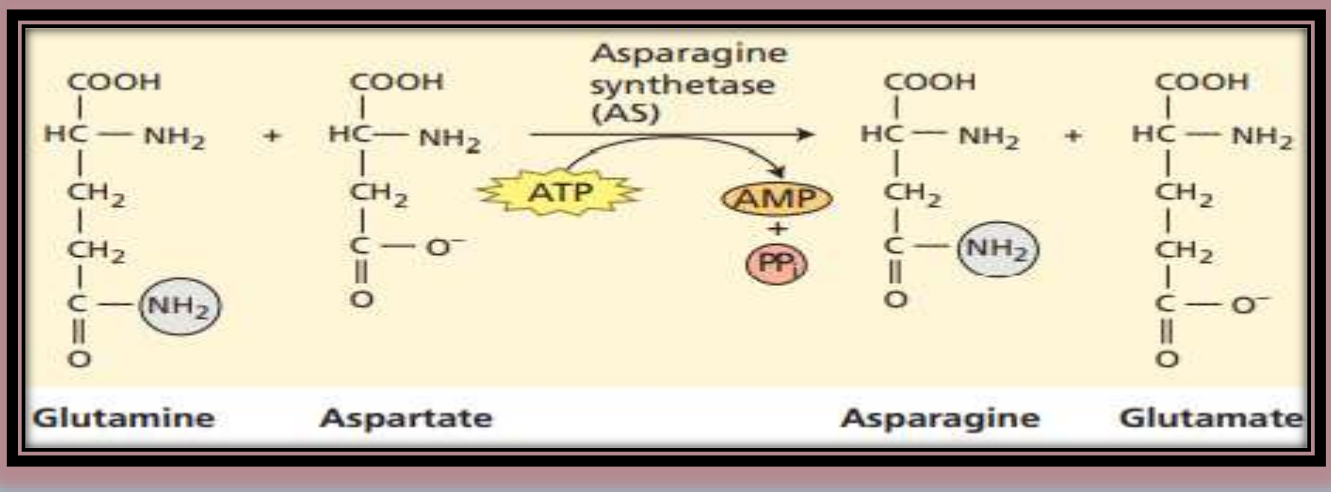

**NOT ALL AMINOACID UNDERGOES TRANS AMINATION REACTION**
**Eg.: Lysine, Threonine, Proline, Hydroxy proline.**




## ASPARAGINE AND GLUTAMINE LINK CARBON AND NITROGEN METABOLISM:

Asparagine, isolated from asparagus as early as 1806, was the first amide to be identified. It serves not only as a component of proteins, but also as a key compound for nitrogen transport and storage because of its stability and high nitrogen-to-carbon ratio (2 N to 4 C for asparagine compared with 2 N to 5 C for glutamine and 1 N to 5 C for glutamate). The major pathway for asparagine synthesis involves the transfer of the amide nitrogen from glutamine to aspartate.

**Glutamine + Aspartate + ATP → Glutamate + Asparagine + AMP + PPi**




High levels of light and carbohydrate—conditions that stimulate plastid GS and Fd-GOGAT—inhibit the expression of genes coding for AS and the activity of the enzyme. The opposing regulation of these competing pathways helps balance the metabolism of carbon and nitrogen in plants. Conditions of ample energy (i.e., high levels of light and carbohydrates) stimulate GS and GOGAT , and inhibit AS; thus they favour nitrogen

assimilation into glutamine and glutamate, compounds that are rich in carbon and participate in the synthesis of new plant materials.

In contrast, energy-limited conditions inhibit GS and GOGAT, stimulate AS, and thus favour nitrogen assimilation into asparagine, a compound that is rich in nitrogen and sufficiently stable for long-distance transport or long term storage.

## AMINO ACID BIOSYNTHESIS

Humans and most animals cannot synthesize certain amino acids—histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, and in the case of young humans, arginine (adult humans can synthesize arginine)—and thus must obtain these socalled essential amino acids from their diet.

In contrast, plants synthesize all of the 20 amino acids that are common in proteins. The nitrogen-containing amino group, as discussed in the previous section, derives from transamination reactions with glutamine or glutamate.

The carbon skeleton for amino acids derives from 3-phosphoglycerate, phosphoenolpyruvate, or pyruvate generated during glycolysis, or from 2-oxoglutarate or oxaloacetate generated in the citric acid cycle . Parts of these pathways required for synthesis of the essential amino acids are appropriate targets for herbicides , because they are missing from animals, so substances that block these pathways are lethal to plants but in low concentrations do not injure animals.

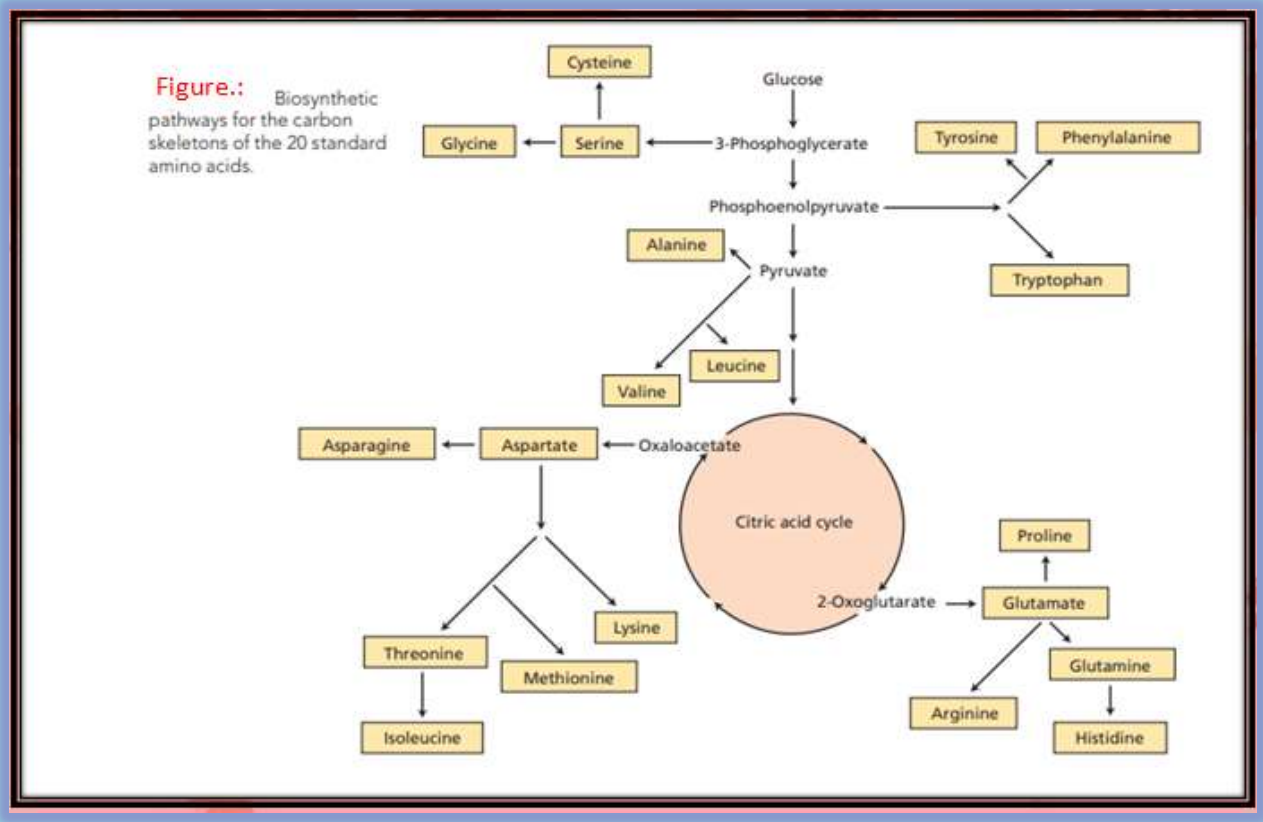



Figure.: Biosynthetic pathways for the carbon skeletons of the 20 standard amino acids.

## Significance

- Formation of non-essential amino acids

  Eg: Alanine , Aspartate
- Recycling of carbon skeletons.

  Eg: Oxaloacetate, Pyruvate

# Conclusion

Ammonium, if it accumulates to high levels in living tissues, is toxic to both plants and animals. Ammonium dissipates transmembrane proton gradients that are required for photosynthetic and respiratory electron transport , for sequestering metabolites in the vacuole , and for

transporting nutrients across biological membranes . Because high levels of ammonium are dangerous, animals have developed a strong aversion to itssmell. The active ingredient in smelling salts, a medicinal vapour released under the nose to revive a person who has fainted, is ammonium carbonate. Plants assimilate ammonium near the site of absorption or generation and rapidly store any excess in their vacuoles, thus avoiding toxic effects on membranes and the cytosol.

In maize (corn; Zea mays) roots, particularly those under potentially toxic levels of ammonia, ammonium may replace glutamine as the source of the amide group.

# Acknowledgement

I would like to express my special thanks and gratitude to Dr. Tripti Roy and Dr. Debjani Sinha Ray, for giving me the golden opportunity to present my topic, for I believe that the researches made on this topic would definitely help me in my future. I would like to extend my sincere regards towards all my respected teachers. I would like to thank my parents and family members who have supported me throughout this whole work. Last but not the least, I would convey my thanks to all my fellow classmates for guiding me , without which this presentation would have been incomplete.

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# Paper-III

# ETHNOBOTANY-Folk Medicines In Ethnobotany

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# Abstract

Ethnobotany- the scientific study of the traditional knowledge and customs of a people concerning plants and their medical, religious, and other uses.

Folk Medicine - medicine using herbal and other remedies based on traditional beliefs. Folk medicines are widely used throughout the world. It originated from primitive man's reaction to natural events and transmitted by a particular community. This types of medicines can be obtained from parts of different types of locally growing plants and herbs like ginger, garlic, gingko etc. They are widely used in the treatment of several diseases like fever, cough, cold, jaundice, malaria, etc. Folk medicines have lot of advantages like low cost, fewer side effects, multiple applications etc. But, alongside it has certain disadvantages too. Still this knowledge of folk medicines must be passed on to the next generations for their preservation.

# Introduction

Ethnobotany is the study of how People of a particular culture or region make use of native (Indeginious) plants.The idea of ethnobotany was first proposed by the early 20$^{th}$ century botanist John William Harshberger.

What is the role of Ethnobotany?

The extract of the plant has been used for centuries in India for treatment of snake-bites, poisoning, hypertension, mental illness and as tranquilizers. The use of this plant in the treatment of different ailments by the tribals or the aboriginals is important as a part of their ethno-medical system.

# APPLICATIONS OF ETHNOBOTANY

- Application of discovering new species- The first and foremost application of Ethnobotany is that it is one of the conventional sources to unravel new species of plants. Botanists can discover new species and further manipulate them to identify their advantages.
- New food sources – Food is the substantial requirement of every human, and we are in an ardent need of discovering new plant species which can fulfil food requirements as per the growing population.
- Application of treating diseases-The best application of Ethnobotany can be considered for treating diseases. It is best because various plants have the astounding potential of treating many conditions and ailments of human beings without imparting any side effects to them.

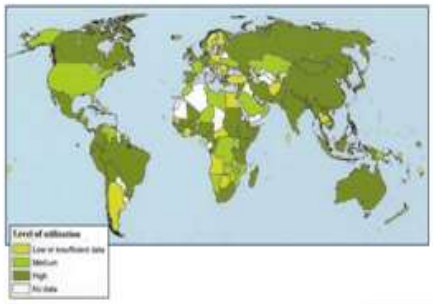
# FOLK MEDICINE

Folk medicines consist of the healing practices and ideas of body physiology and help preservation known to some in a culture having prior experience. It is also known as **Traditional medicine or Indeginious medicine**

A major component of folk medicine is **Herbal Medicine** which is a use of natural plant substance to treat or prevent illness.

# WIDESPREAD USE OF FOLK MEDICINE AROUND THE WORLD

**Use of Herbal Medicine**

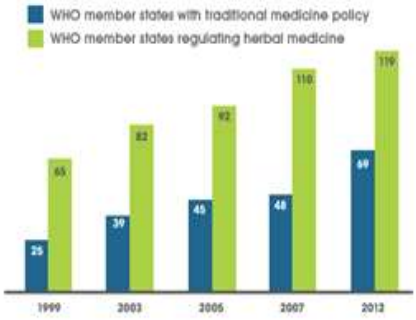


Source: WHO Global Atlas of Traditional, Complementary and Alternative Medicine, 2005.

Traditional medicine is contrasted with scientific medicine. In some Asian and African countries, up to 80% of the population relies on traditional medicine for their primary health care needs. When adopted outside its traditional culture, traditional medicine is often considered a form of alternative medicine.

In a study it is found that in whole world, more than 50000 plant species are identified to have medicinal value ( More than 70% of Indian population depends on herbal treatment and over 6000 of 15000 herbal plant species have been used as herbal drugs or medicine by herbal medical practitioners in India

Figure 1: **Progress in worldwide regulation of traditional medicine**




# ORIGIN AND TRANSMISSION OF THE KNOWLEDGE OF FOLK MEDICINE

**ORIGIN :** Folk or traditional medicine originated from primitive man's reaction or attitude to natural events, people's effort to find solutions to the diseases set up the basis of folk medicine.

## TRANSMISSION OF KNOWLEDGE:

Folk medicine is generally transmitted orally through a community, family and individuals until collected within a given culture, elements of Indeginious medicine knowledge maybe diffusely known by many, or maybe gathered and applied by those in a specific role of healer such as a"SHAMMAN" or "MIDWIFE". Three factors that legitimise the role of the healer:-

- Their own beliefs
- The success of their actions
- The beliefs of the community.

Since time immemorial tribal communities have preserved and maintained their traditional and Indeginious knowledge of medicinal plants and animals every tribal group has a unique and specific knowledge of Ethnomedicinal practices that differs from other tribal groups.

# EXAMPLES OF SOME FOLK MEDICINES

TRADITIONAL CHINESE MEDICINE

Traditional Chinese Medicine, Traditional Korean Medicine, Arabic Indeginious Medicine, Japanese Kampo Medicine, Traditional Aboriginal Bush Medicine, Georgean Folk Medicine, among others.

TRADITIONAL KOREAN MEDICINE

JAPANESE KAMPO MEDICINE

ARABIC INDEGINIOUS MEDICINE

TRADITIONAL ABORIGINAL BUSH MEDICINE

# HOW THE FOLK OR TRADITIONAL MEDICINE IS DIFFERENT FROM THAT OF MODERN OR BIO- MEDICINE

## FOLK MEDICINE

- Folk Medicine accepts the existence of germs, explains the disease by magical and supernatural events
- The knowledge is oral not written and is limited to few local, specialized, tribal medical practitioners called 'Shaman'. Ethnomedicine or Folk Medicine allows use of medical plants and animal products and magical practices for curing the disease.
- Folk Medicine is more ethnic, more magical and less scientific. It is the study of healing practices of cultural

## MORDERN MEDICINE

- Mordern Medicine tries to explain the cause of the disease by germ theory
- Mordern Medicine is in written form, is global and universal and there is neither any use of meat or flesh of animals nor any performance of religio-magical rites for the treatment of diseases.
- Biomedicine or Mordern Medicine is based on pure scientific knowledge and techniques and it can be

- Groups and individuals experiences disease and illness.
- Folk Medicines are widely effective for general cough, cold, gastric or liver problems and joint pains etc. But are not that effective for severe problems like cancer, diabetes, blood pressure etc.

- Effective for cute/chronic/deadly diseases
- Mordern Medicines are effective for all type of diseases both acute and chronic.

# SOME IMPORTANT HERBS OR PLANTS AND THEIR PARTS WHICH ARE USED TO MAKE FOLK MEDICINES

- **CHAMOMILE** ( FLOWER) Chamomile for the skin (topical) may be used to treat skin irritation from radiation cancer treatments. Chamomile in capsule form may be used to control vomiting during chemotherapy.

- **ECHINACEA** (LEAF,STALK,ROOT) Echinacea is commonly used to treat or prevent colds, flu, and infections, and for wound healing.


- **FEVERFEW** (LEAF) Feverfew was traditionally used to treat fevers. It is now commonly used to prevent migraines and treat arthritis. Some research has shown that certain feverfew preparations can prevent migraines. Side effects include mouth ulcers if the leaves are chewed and digestive irritation.

- **GARLIC** (CLOVES,ROOT) Garlic has been used all over the world in cooking and for its many medicinal properties. The compounds isolated from garlic have been shown to have antimicrobial, cardioprotective, anticancer and anti-inflammatory properties. These properties may play a role in the belief that garlic helps lower cholesterol and blood pressure.


- **GINGER** (ROOT) Ginger is most commonly known as an herb for easing nausea and motion sickness. Research suggests that ginger may relieve the nausea caused by pregnancy and chemotherapy. It's wide range of actions may be due in part to its strong anti-inflammatory and antioxidative effects.

- **GINGKO** (LEAF) Ginkgo leaf extract has been used to treat a variety of conditions such as asthma, bronchitis, fatigue, and tinnitus. It is also used to improve memory and to prevent dementia and other brain disorders.

- **GINSENG ( ROOT)** Ginseng is used as a tonic and aphrodisiac, even as a cure-all.

- **GOLDENSEAL** (ROOT, RHIZOME) Goldenseal is used to treat diarrhea and eye and skin irritations. It is also used as an antiseptic. It is also an unproven treatment for colds.

- **VALERIAN** (ROOT) Valerian is used to treat sleeplessness and to reduce anxiety. Research suggests that valerian may be a helpful sleep aid, but the evidence is not consistent to confirm it.

# SOME APPLICATIONS OF FOLK MEDICINE

•COUGH: a.The patient drinks a spoonful of honey mixed with a spoonful of lemon juice every morning for a few days.

b. The patient eats raw parsley.

- FLU: Mint and dried linden flowers are bolied with Lemon and drunk as tea.
- SNAKE BITES : The head of a match is scraped and this is rubbed on the affected part.
- MALARIA : A small herb with pink flowers known as “malaria weed” is boiled and drunk as tea.
- JAUNDICE : The patient’s forehead or chest is scratched with a razor blade.
- HAIR LOSS : For healthy hair and to avoid baldness, vine stems are chopped in the spring time. The liquid that drips from these stems is collected in a bottle and the hair washed with it.

- SWOLLEN STOMACH : A mixture of vinegar and bran is heated, and the stomach covered with the mixture.
- DIARHOEA : a) Diarrhea will end if a glass of soda pop with an asprin inside is drunk.

b) A spoonful of coffee is mixed with lemon juice and eaten.

- HIGH TEMPERATURE: a) A towel is moistened with vinegar and pressed onto the brow, neck, hands, feet and the whole body. This operation is repeated until the patient's temperature gone down.
- ASTHMA: A pigeon egg is consumed every morning for 40 days as the first meal of the day.
- ACHES: a) The leaf of a black cabbage is heated and placed on the affected area. This operation is repeated frequent.
- BRONCHITIS : a) Linen seeds are mashed with sugar and eaten.

b) A piece of bread is roasted, moistened with vinegar and placed on chest.

- TONSILITIS : The throat is covered with a piece of cotton with pepper and grain alcohol.
- NOSE BLEEDS : The shell of an egg is burned till it turn into ashes. The victim breathes in this ash when his or her nose starts to bleed.
- EAR ACHE : A little leek water is poured into the ear.
- DOG BITES : The bite is covered with a bread poultice.
- STOMACH ACHE : a) The patient drinks milk with honey.

  b) Inula is boiled and drunk as tea.

- SHORTNESS OF BREATH : a) Stingling nettle tea is drunk every day.
- CANCER : In summer fresh and in winter dry stinging nettles are boiled and drunk as tea every morning before breakfast.

## ADVANTAGES OF FOLK MEDICINE

- **THEY ARE OF LOW COST** – the rising cost of prescription drugs have led the people to look for alternatives. While medicinal herbs may not be as strong or as fast acting as conventional medicine, there is a growing body of scientific evidence that shows their efficacy and in what doses.
- **THEY MAY HAVE FEWER SIDE EFFECTS** – While the side effects of any herbal medication depend on the drug in question, may have fewer side-effects than the conventional medicine.
- **THERE IS A CHOICE ON HOW TO USE THEM** – medicinal herbs can be used in a variety of ways, depending on the kind of herbs that is to be used. Some can be fixed with food, some can be made into tea, and there are some which can be available in capsule form.
- **THEY ARE GOOD FOR MORE THAN ONE CONDITION** – most prescriptive drugs are designed for a specific health problem. By contrast, many herbal medicine act on several parts of the body at once.

## DISADVANTAGES OF FOLK MEDICINE

- Folk medicines take too much time to act, and the the entire process is very slow.
- They contain various ingredients which sometimes causes allergic reactions.
- Folk medicines are not good for serious cases such as heart attack and broken bones. These medicines are also ineffective in sudden illnesses and accidents. Folk medicines for disorders may have negative side effects, which sometimes takes a long time to reveal.
- Herbs used for making folk medicine harvested in the wild are risky. Incorrect identification of the required herb can even lead to poisoning.

# Conclusion

Ethnomedicine or Folk medicine is a traditional knowledge which should be preserved as it is thought to be vanished in the near future. Folk medicine will be a good option for future generations as it is a technique which involves sustainable use of natural resources which is very important for life on planet earth. Folk Medicine is such a knowledge of old people mostly tribals which utilizes nature in balance for the welfare of society by treating their diseases and ailments with the

help of herbs and medicinal animals. Folk medicine is the cultural heritage of the tribal people in which they heal and cure the disease by using some zoo-botanical products and practising magicoreligious rituals.

# Acknowledgement

I would like to extend my gratitude towards my teachers Dr. Tripti Roy and Dr. Debjani Singha Roy for providing me this opportunity of giving a presentation on this topic. I would also like to thank all my departmental teachers for teaching me and thus making me well informed about this particular topic. Last but not the least I would like to thanks all my fellow classmates for bearing with me in this entire period of time.

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Paper-IV

# CRASSULACEAN ACID METABOLISM (CAM) PATHWAY


*By*

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# Abstract

Photosynthetic adaptation which is found in succulent plants is Crassulacean acid metabolism (CAM). CAM is a mechanism for concentrating Carbondioxide around RuBisCo (Ribulose-1,5-bisphosphate carboxylase/oxygenase) in plants that inhabit environments with seasonal water availability. The pathway can be attributed to the cyclical process of acidification and deacidification through day and night. Carbon di oxide is stored as Malate in the vacuole of cell during day and Malate is decarboxylated to produce CO2 for Calvin cycle during night. By this mechanism CAM plants effectively conserve water.

# Introduction

❖ **What is CAM?**

- ❑ Specialised photosynthetic process
- ❑ Variant of $C_4$ cycle
- ❑ Mechanism for concentrating $CO_2$ around RuBisCo in plants having seasonal water availability

➢ First observed in *Bryophyllum calycinum* , a succulent member of the Crassulaceae

❖ **Characteristics of CAM plant:**

- ❑ Thick cuticles
- ❑ Large vacuoles
- ❑ Stomata with small apertures
- ❑ Sunken stomata

Euphorbiaceae, Orchidaceae, Liliaceae are the other examples of Crassulacean acid metabolism.

❖ **Types of CAM plants**

- ❑ Constitutive or obligate CAM plants :
  - ✓ nocturnal uptake of $CO_2$ all times
  - ✓ Eg- *Opuntia basilaris* (fig-A)
- ❑ Facultative CAM plants :
  - ✓ Uses CAM pathway only under water or salt stress
  - ✓ Eg- *Mesembryanthemum crystallinum* (fig-B)

❖ fig-A

❖ fig-B

❖ **Phases of CAM**

- ❑ **Phase I**
  - ✓ Nocturnal uptake of atmospheric $CO_2$
  - ✓ Open stomata
- ❑ **Phase II**
  - ✓ Early morning time
  - ✓ RuBisCo activity starts increasing
- ❑ **Phase III**
  - ✓ Diurnal phase
  - ✓ Closed stomata
  - ✓ Photosynthesis occurs
- ❑ **Phase IV**
  - ✓ Late afternoon time
  - ✓ RuBisCo activity decreases
  - ✓ PEPCase activity starts increasing


❖ Fig : CAM circadian rhythm

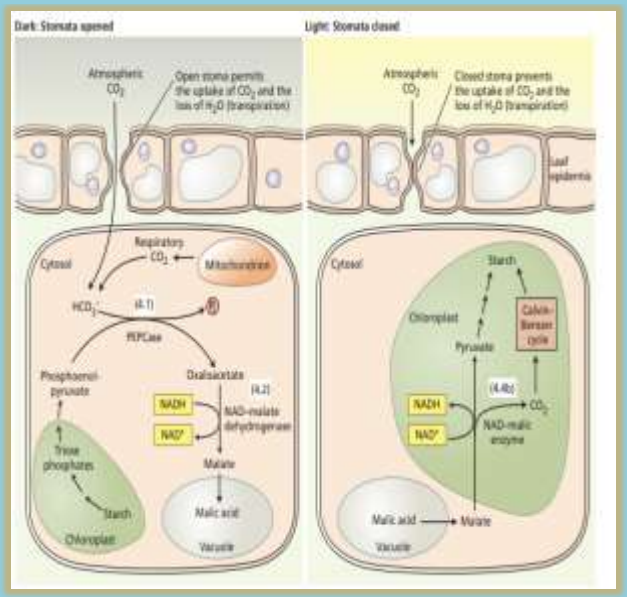



**Fig:Mechanism of CAM pathway**

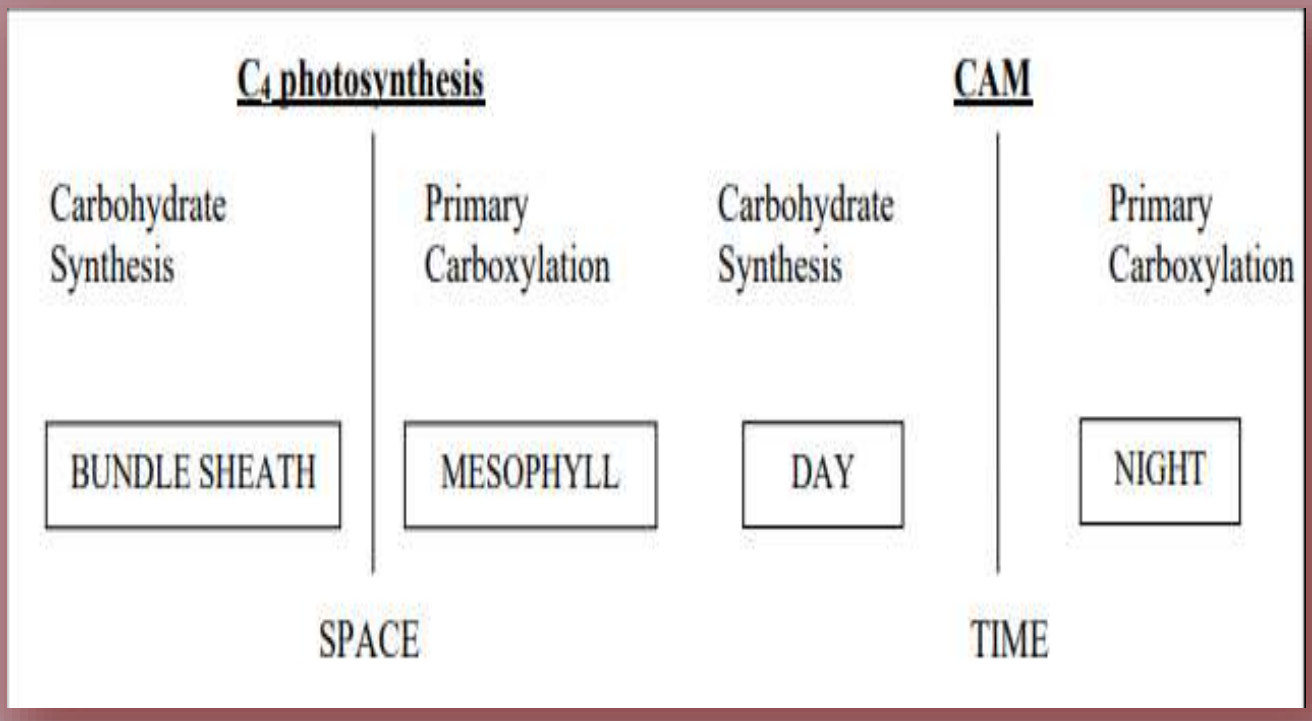
## ❖ Acidification :

- ✓ Accumulation of malic acid
- ✓ Phosphoenol pyruvate + $HCO_3^-$ $\xrightarrow{\text{PEP Carboxylase}}$ Oxaloacetate + $P_i$
- ✓ Oxaloacet*ate* + $NADH + H^+$ $\xrightarrow{\text{NAD Malate dehydrogenase}}$ Malate + $NAD^+$

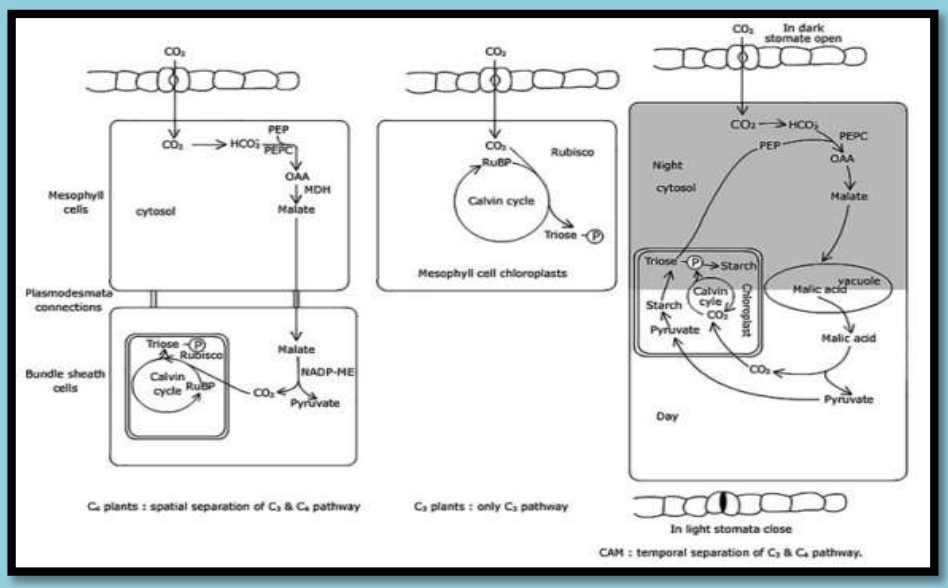
## ❖ Deacidification :

- ✓ Decarboxylation of malate
- ✓ Mal*ate* + $NAD^+ \longrightarrow$ Pyruvate + $CO_2$ + NADH + $H^+$

## ❖ Relationship between $C_4$ and CAM :



# ❖ Comparison of $C_3$, $C_4$ and CAM




## ❖ Significance of CAM :

- ✓ High Water use efficiency (WUE)
  - ➢ Water use efficiency = moles of $CO_2$ assimilated / moles of $H_2O$ transpired
- ✓ Low transpiration ratio
  - ➢ Transpiration ratio = 1 / WUE
- ✓ Idling CAM

# Conclusion

CAM is a physiological process to concentrate co2 around RuBisCo in plants with seasonal water scarcity. By this specialized photosynthetic process plants reduce water loss by decreasing transpiration.

# Acknowledgement

I would like to express my gratitude to Dr.Tripti Roy and Dr.Debjani Sinha Ray, associate professors of the Department of Botany, Bethune College, Kolkata, for giving me this opportunity to present this topic. Thank you for your support and guidance.

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Paper-v

# BETA OXIDATION OF FATTY ACIDS

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# Abstract

Beta oxidation is a basic process of fatty acid breakdown leading to gradual production of energy. For it to take place there are certain required conditions. It requires the activation of free fatty acids. The entire process involves five basic steps which goes on in a cyclic manner until the complete oxidation of the given fatty acid chain has occurred. It occurs both in mitochondria and peroxisome with some basic differences. The stoichiometry reveals that beta oxidation of fatty acids produces more amount of energy than the normal respiratory process. There are also means of regulating the process, all of which have been discussed thoroughly in the given presentation.

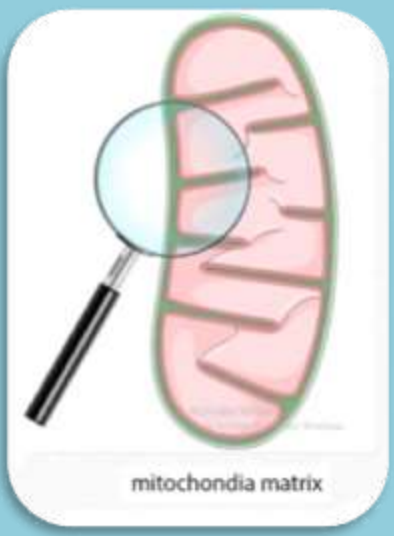
# Introduction

Beta- oxidation is the process by which fatty acid molecules are down in the mitochondria to generate Acetyl-coA, which enters citric acid cycle, and NADH and are produced, which are used by electron transport chain.

## WHERE DOES BETA OXIDATION TAKES PLACE?

- **Fatty acid breakdown occurs in the cytosol of prokaryotes.**
- **In the mitochondrial matrix of all other eukaryotes.**

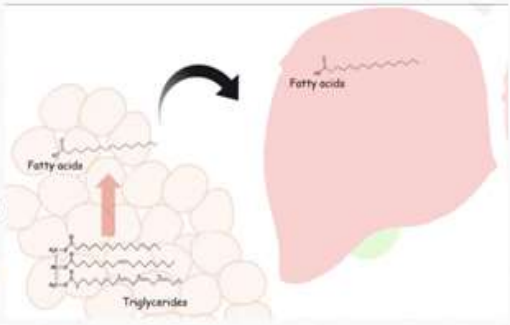
- **In peroxisomes in plants.**


## Under what conditions Beta-oxidation takes place?

- The metabolic state is the key aspect or key circumstance under which fatty acid oxidation is favored.
- For example, under the Fed stage when our body has too much energy then fatty acid anabolism is prefer. In this state , the body would prefer to store the fat in the adipose tissue.
- But in fasted state, the fats would be broken down and it would be utilized to generate energy.

- **What happens in the fasting state!?**

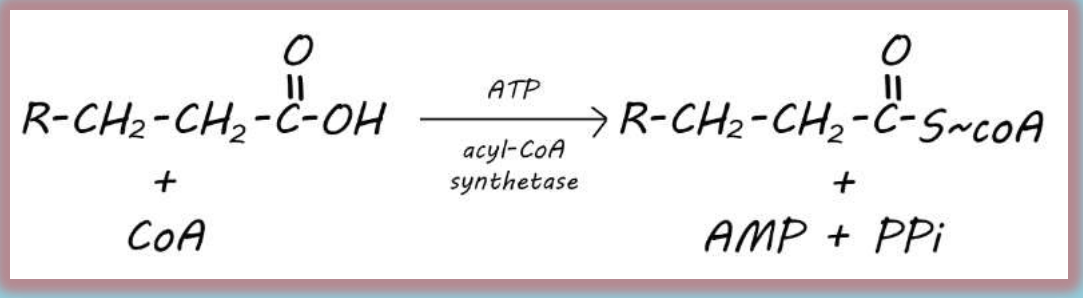



- In the fasted state, inside the adipose tissue, the stored triglycerides would be broken to produce Free Fatty Acid (FFA).
- Now these FFA would be carried to the liver and in the liver hepatocytes inside the mitochondria they would be broken down and they would be oxidized.
- The ultimate goal is to generate energy, under the circumstances of fasting. Because in fasting we don't have the supply of food so glucose level is limiting

# Fatty acid Activation

- **Free Fatty Acids (FFA) must first be converted to an active intermediate before they can be catabolized.**

- **The relative stability of the C—C bonds in a fatty acid is overcome when, the carboxyl group at C-1 is activated by attachment to coenzyme A, which allows stepwise oxidation of the fatty acyl group.**

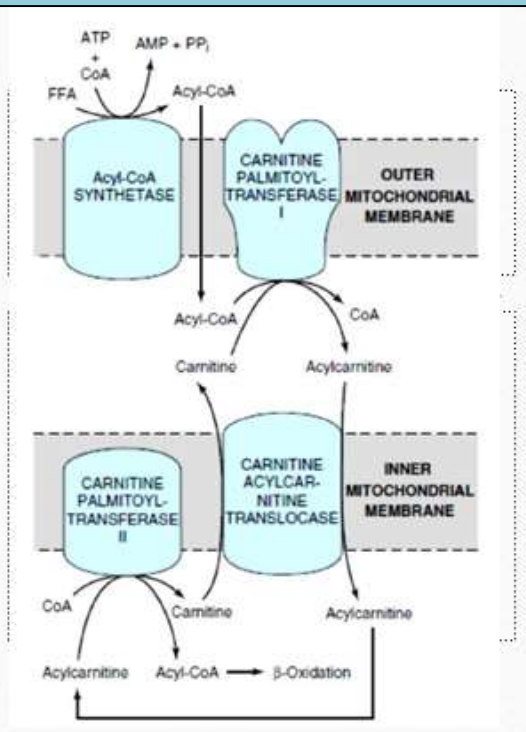



- This is the only step in the complete degradation of a fatty acid that requires energy from ATP.

- In the presence of ATP and coenzyme-A, the enzyme acyl-CoA synthetase (thiokinase) catalyzes the conversion of a fatty acid or FFA to an "active fatty acid" or acyl-CoA, which uses one high-energy phosphate with the formation of AMP and PPi.
- The PPi is hydrolyzed by inorganic pyrophosphate with the loss of a further high-energy phosphate, ensuring that all overall reaction goes to completion.

## How does Fatty Acid enter Mitochondria?

- The enzymes of fatty acid oxidation in animal cells are located in the mitochondrial matrix, as demonstrated in 1948 by Eugene P. Kennedy and Albert Lehninger.
- The fatty acids with chain lengths of 12 or fewer carbons enter mitochondria without the help of membrane transporters.
- Those with 14 or more carbons, which constitute the majority of the FFA obtained in the diet or released from adipose tissue, cannot pass directly through the mitochondrial membranes—they must first undergo the three enzymatic reactions of the **carnitine shuttle**.



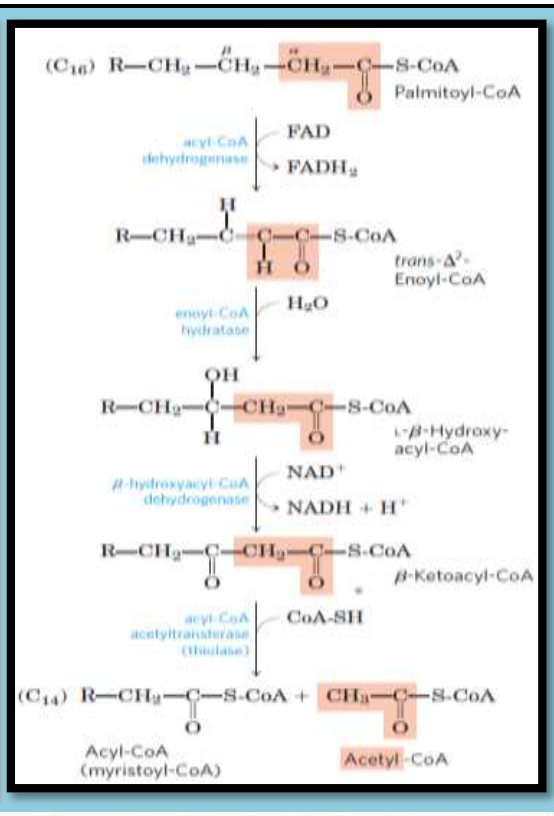

- **Carnitine palmitoyl transferase-I**, present in the outer mitochondrial membrane, converts long chain acyl-coA to acylcarnitine, which is able to penetrate the inner membrane and gain access to the β-oxidation system of enzyme.
- **Carnitine acylcarnitine translocase** acts as an inner membrane exchange transporter. It transfers the acylcarnitine from intermembrane space to inner membrane of mitochondria.
- The acylcarnitine then reacts with CoA, catalyzed by **Carnitine palmitoyl transferase-II**, located on the inside of inner membrane. Acyl-CoA is reformed in the mitochondrial matrix, and carnitine is liberated.

← (As shown in this figure)

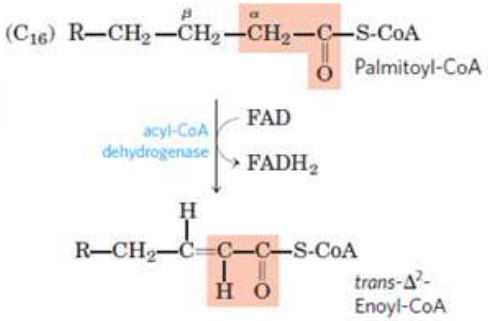
# β- Oxidation

β- Oxidation of saturated fatty acids have four basic steps

1. **α, β dehydrogenation** of the acyl CoA to form a *trans*- Δ²-enoyl CoA;
2. **Hydration of α, β unsaturated acyl-CoAs** to form β -hydroxyacyl-CoA;
3. **Oxidation of β -hydroxyacyl-CoA** to form β-ketoacyl CoA;
4. **Thiolysis** by a second CoA molecule to form acetyl CoA and an acyl CoA shortened by two carbon atoms.

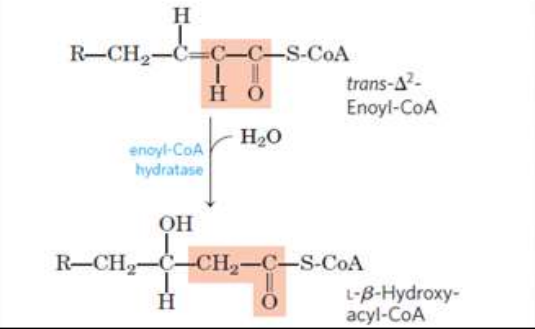



## STEP 1- α, β dehydrogenation of the acyl CoA



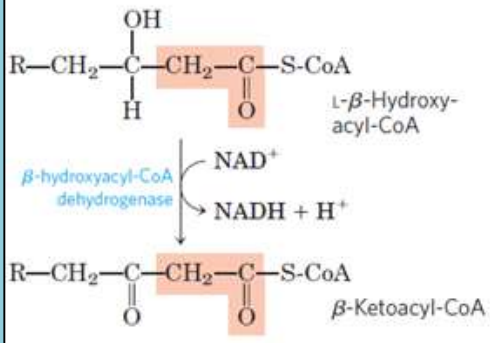

- This step is catalysed by three isozymes of acyl-CoA dehydrogenase, each specific for a range of fatty-acyl chain length-
  - very-long-chain acyl-CoA dehydrogenase (VLCAD) acting on fatty acids of 12-18 carbon
  - medium-chain acyl-CoA dehydrogenase (MCAD) acting on fatty acids of 4-14 carbon
  - short-chain acyl-CoA dehydrogenase (SCAD) acting on fatty acids of 4-8 carbon
- The electrons removed from the fatty acyl–CoA are transferred to FAD, and the reduced form of the dehydrogenase immediately donates its electrons to an electron carrier of the mitochondrial respiratory chain, the **electron-transferring flavoprotein (ETF).**
- FAD is the electron acceptor, and electrons from the reaction ultimately enter the respiratory chain and pass to O2, with the concomitant synthesis of about 1.5 ATP molecules per electron pair.

## STEP 2- Hydration of α, β unsaturated acyl-CoAs



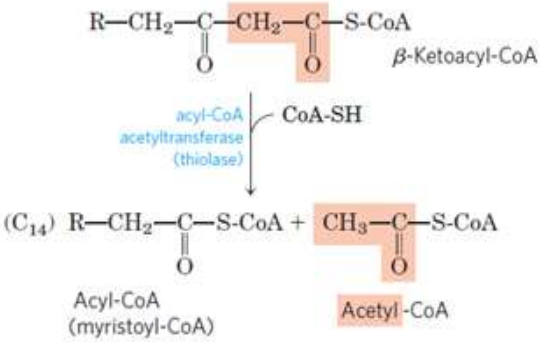

- In the second step of the -oxidation cycle, water is added to the double bond of the *trans*-Δ²-enoyl-CoA to form the L stereoisomer of **β -hydroxyacyl-CoA (3-hydroxyacyl-CoA).**
- This reaction, catalyzed by **enoyl-CoA hydratase**, is formally analogous to the fumarase reaction in the citric acid cycle, in which H2O adds across an – double bond

## STEP 3- Oxidation of β -hydroxyacyl-CoA

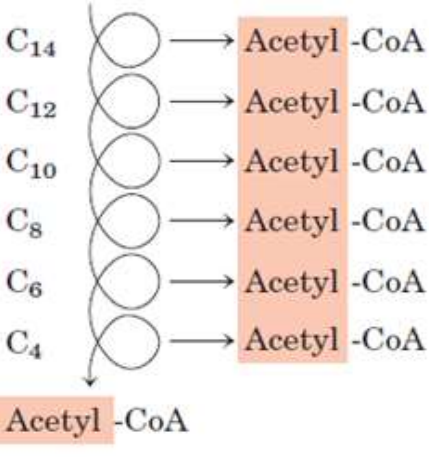



- In the third step, L- β -hydroxyacyl-CoA is dehydrogenated to form **β -ketoacyl-CoA**, by the action of **β -hydroxyacyl-CoA dehydrogenase**;
- NAD+ is the electron acceptor.
- This enzyme is absolutely specific for the L stereoisomer of hydroxyacyl-CoA.
- The NADH formed in the reaction donates its electrons to **NADH dehydrogenase**, an electron carrier of the respiratory chain, and ATP is formed from ADP as the electrons pass to $O_2$.
- The reaction catalyzed by **β -hydroxyacyl- CoA** dehydrogenase.

## STEP 4- Thiolysis or thioclastic scission



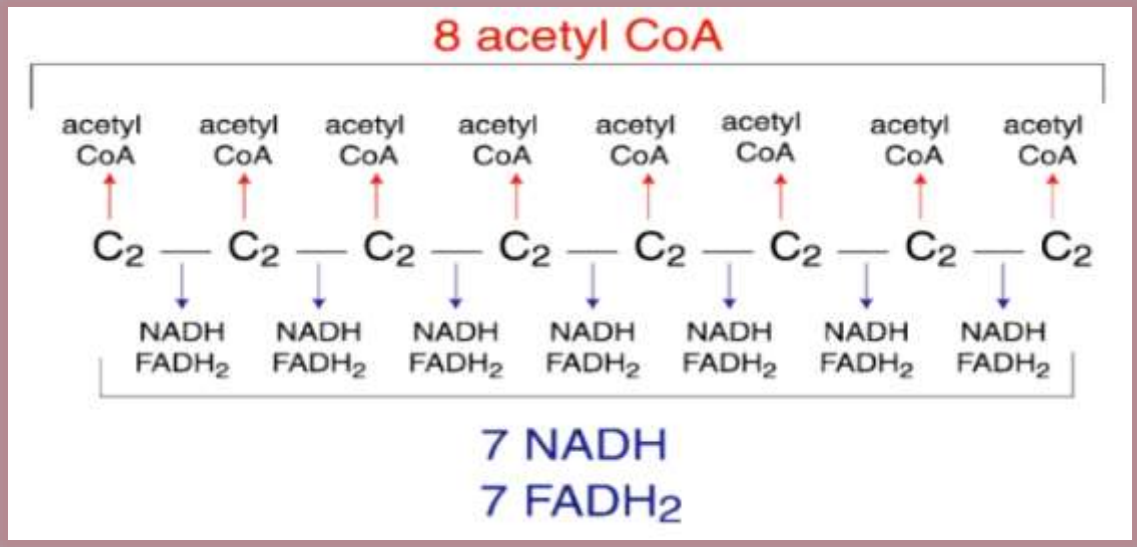

- The fourth and last step of the -oxidation cycle is catalyzed by **acyl-CoA acetyltransferase**, more commonly called **thiolase**, which promotes reaction of β -ketoacyl-CoA with a molecule of free coenzyme A to split off the carboxyl-terminal two-carbon fragment of the original fatty acid as acetyl-CoA.
- The other product is the coenzyme A thioester of the fatty acid, now shortened by two carbon atoms.
- This reaction is called thiolysis, by analogy with the process of hydrolysis, because the -ketoacyl-CoA is cleaved by reaction with the thiol group of coenzyme A.




- The myristoyl-CoA can now go through another set of four β–oxidation reactions, exactly analogous to the first, to yield a second molecule of acetyl-CoA and lauroyl-CoA, the coenzyme A thioester of the 12-carbon laurate.
- Altogether, seven passes through the β -oxidation sequence are required to oxidize one molecule of palmitoyl-CoA to eight molecules of acetyl-CoA.
- The overall equation is

$$\text{Palmitoyl-CoA} + 7\text{CoA} + 7\text{FAD} + 7\text{NAD}^+ + 7\text{H}_2\text{O} \longrightarrow 8\ \text{acetyl-CoA} + 7\text{FADH}_2 + 7\text{NADH} + 7\text{H}^+$$

# Stoichiometry of Beta oxidation




- For each acetyl-CoA entering the Krebs cycle 10ATPs are produced.
- Each molecule of FADH2 formed during oxidation of the fatty acid donates a pair of electrons to ETF of the respiratory chain, and about 1.5 molecules of ATP are generated during the ensuing transfer of each electron pair to O2.
- Similarly, each molecule of NADH formed delivers a pair of electrons to the mitochondrial NADH dehydrogenase, and the subsequent transfer of each pair of electrons to O2 results in formation of about 2.5 molecules of ATP.

1. No. of Acetyl-CoA formed = 16/2= 8
Each Acetyl-CoA enters Kreb's cycle, so ATP produced
8x10=80 ATPs

2. NADH formed = 16/2-1 = 7
therefore, ATP formed = 7x2·5= 17·5 ATPs

3. FADH2 formed = 16/2-1 = 7
therefore, ATP formed = 7x1·5 = 10·5 ATPs

Therefoe, total ATP formed = 80+17·5+10·5 = 108 ATPs

**TABLE 17–1** Yield of ATP during Oxidation of One Molecule of Palmitoyl-CoA to $CO_2$ and $H_2O$

| Enzyme catalyzing the oxidation step | Number of NADH or $FADH_2$ formed | Number of ATP ultimately formed* |
|---|---|---|
| Acyl-CoA dehydrogenase | 7 $FADH_2$ | 10.5 |
| β-Hydroxyacyl-CoA dehydrogenase | 7 NADH | 17.5 |
| Isocitrate dehydrogenase | 8 NADH | 20 |
| α-Ketoglutarate dehydrogenase | 8 NADH | 20 |
| Succinyl-CoA synthetase | | 8† |
| Succinate dehydrogenase | 8 $FADH_2$ | 12 |
| Malate dehydrogenase | 8 NADH | 20 |
| Total | | 108 |

*These calculations assume that mitochondrial oxidative phosphorylation produces 1.5 ATP per $FADH_2$ oxidized and 2.5 ATP per NADH oxidized.

†GTP produced directly in this step yields ATP in the reaction catalyzed by nucleoside diphosphate kinase (p. 526).

The following table summarizes the yield of NADH, FADH2 and ATP in the successive steps of palmitoyl-CoA oxidation.

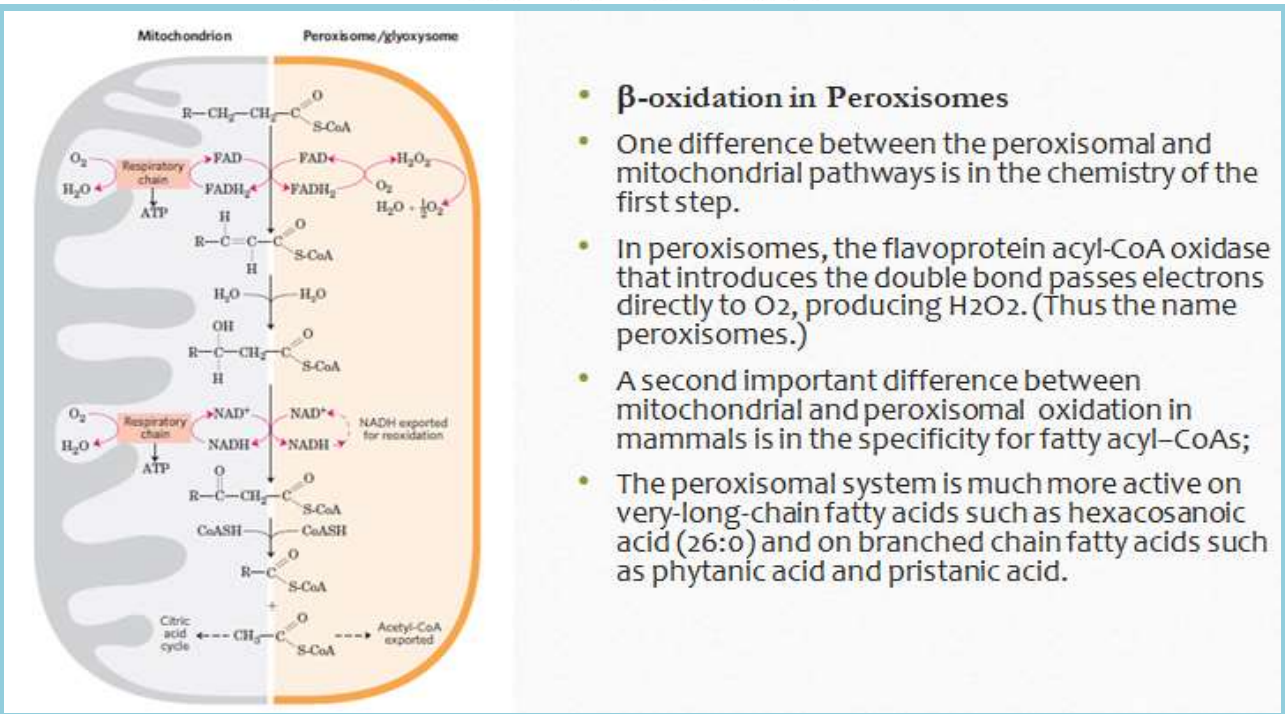
Two high-energy phosphate bonds (phosphoanhydride bonds) are cosumed in the activation of palmitate to palmitoyl-CoA, in which ATP is split into AMP and 2Pi by inorganic pyrophosphatase (ATP -> AMP+ PPi & PPi -> 2Pi)

The overall reaction is:

Fatty acid + CoA + 2ATP -> Fatty acyl-CoA + 2AMP + 4PPi

(palmitate) (palmitoyl-CoA)

Thus, the net yield from the complete oxidation of one molecule of palmitate is 108 - 2 = 106 ATPs




- **β-oxidation in Peroxisomes**
- One difference between the peroxisomal and mitochondrial pathways is in the chemistry of the first step.
- In peroxisomes, the flavoprotein acyl-CoA oxidase that introduces the double bond passes electrons directly to O2, producing H2O2. (Thus the name peroxisomes.)
- A second important difference between mitochondrial and peroxisomal oxidation in mammals is in the specificity for fatty acyl–CoAs;
- The peroxisomal system is much more active on very-long-chain fatty acids such as hexacosanoic acid (26:0) and on branched chain fatty acids such as phytanic acid and pristanic acid.

# Regulation of β-oxidation

- Malonyl-CoA can act to prevent fatty acyl CoA derivatives from entering the mitochondria by inhibiting the carnitine acyl transferase that is responsible for this transport.
- When fatty acyl-CoA levels rise, β-oxidation is stimulated.
- Increased citrate levels; however, inhibit β-oxidation.
- Because this reflects an abundance of acetyl-CoA, it too inhibits β-oxidation.

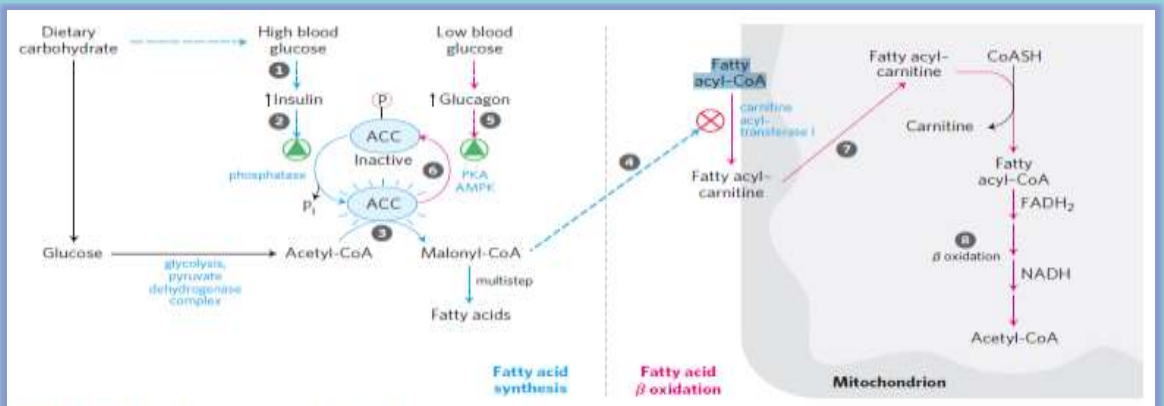



**FIGURE 17–13 Coordinated regulation of fatty acid synthesis and breakdown.** When the diet provides a ready source of carbohydrate as fuel, β oxidation of fatty acids is unnecessary and is therefore down-regulated. Two enzymes are key to the coordination of fatty acid metabolism: acetyl-CoA carboxylase (ACC), the first enzyme in the synthesis of fatty acids (see Fig. 21–1), and carnitine acyltransferase I, which limits the transport of fatty acids into the mitochondrial matrix for β oxidation (see Fig. 17–6). Ingestion of a high-carbohydrate meal raises the blood glucose level and thus ❶ triggers the release of insulin. ❷ Insulin-dependent protein phosphatase dephosphorylates ACC, activating it. ❸ ACC catalyzes the formation of malonyl-CoA (the first intermediate of fatty acid synthesis), and ❹ malonyl-CoA inhibits carnitine acyltransferase I, thereby preventing fatty acid entry into the mitochondrial matrix. When blood glucose levels drop between meals, ❺ glucagon release activates cAMP-dependent protein kinase (PKA), which ❻ phosphorylates and inactivates ACC. The concentration of malonyl-CoA falls, the inhibition of fatty acid entry into mitochondria is relieved, and ❼ fatty acids enter the mitochondrial matrix and ❽ become the major fuel. Because glucagon also triggers the mobilization of fatty acids in adipose tissue, a supply of fatty acids begins arriving in the blood.

# Conclusion

Fatty acid β-oxidation is major metabolic pathway that is responsible for the mitochondrial breakdown of long-chain acyl-CoA to acetyl-CoA. This process involves many steps that are regulated at the transcriptional and post-transcriptional level.

Transcriptional regulation involves PPARs, SREBP1, and PGC-1α, while the post-transcriptional level mainly involves allosteric control of fatty acid β–oxidation, as well as ACC, MCD, and CPT regulation. Both mechanisms work in harmony to ensure a continual supply of long-

chain acyl-CoA for β-oxidation, and products of β-oxidation for mitochondrial energy production.

# Acknowledgement

I would like to express my special thanks and gratitude to my teachers Dr. Tripti Roy and Dr. Debjani Sinha Roy who gave me the golden opportunity to do a presentation on the topic β-oxidation, which also helped me in doing a lot of Research and I came to know about so many new things. I am really thankful to them.

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# Paper-VI

# PHOTORESPIRATION IN PLANTS

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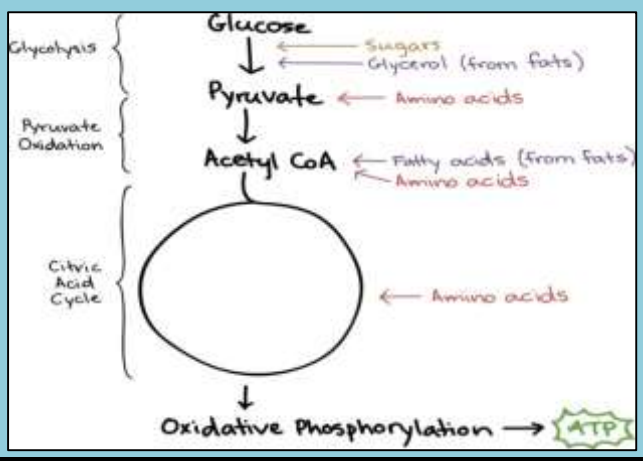
# Abstract

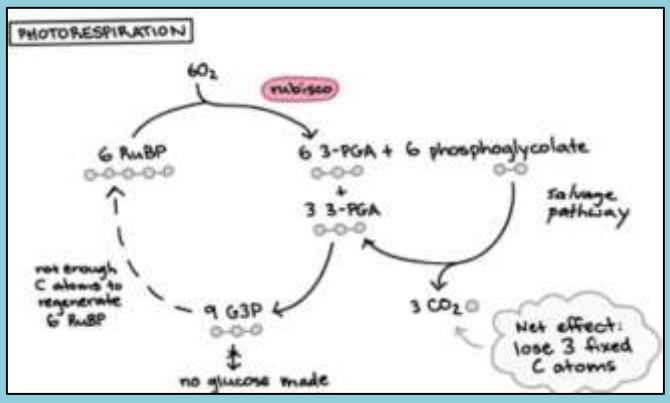
Photorespiration in plants is the light dependent oxygen fixation process of organic substrates along with their decarboxylation, without producing ATP. In plants its occurrence involves 3 subcellular components- the chloroplast, peroxisome and mitochondria. It is ancillarily related with photosynthesis, where initially it eliminates carbon , destined to be fixed by the Calvin-Benson cycle, but later on recovers 75% of it. Ribulose-1,5-bisphosphate is oxygenated by enzyme Ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCo) forming 2- phosphoglycolate that undergoes the photorespiratory cycle (C2 cycle) to regenerate 3- phosphoglycerate , the stable intermediate of Calvin cycle. The C2 cycle provides substrates for several metabolic pathways, protects plants from photoinhibition, and is significant in plants like soybean,wheat, etc. Though there is a confusion about it being wasteful or useful for plants. In this presentation I've tried to explain different aspects of the photorespiratory cycle in plants and the enzymes controlling it.

# Introduction

**<u>Respiration</u> is a metabolic process, in which free energy released from the oxidation of organic compounds to $CO_2$ and $H_2O$ is used to generate ATP.**



Photorespiration is a process of light dependent uptake of $O_2$ Concomitant to the release of $CO_2$ from organic compounds, without any ATP generation. This process Metabolically and functionally linked to photosynthesis.




CARBON FIXATION REACTIONS

- C3 CYCLE ( Calvin – Benson cycle)
- C4 CYCLE ( Hatch –Slack cycle)
- CAM PATHWAY

OXYGEN FIXATION REACTION

- C2 OXYDATIVE PHOTOSYNTHETIC CARBON CYCLE (PHOTORESPIRATION ) or PHOTOSYNTHETIC CARBON OXIDATION (PCO) CYCLE.

- Photorespiration and carbon fixing reactions occurs simultaneously but in diametrically opposite direction.
- Thus the former is an ancillary component of Photosynthesis that salvages some of the assimilated carbon , while the later fixes $CO_2$ .
- Photorespiration involves 3 subcellular compartments- the **Chloroplast** , the **Peroxisome**, and the **Mitochondria** .

# SHORT HISTORY

- Otto Heinrich Warburg (1920) , observed that photosynthesis was inhibited by oxygen in *Chlorella* . – WARBURG 'S EFFECT

- Decker observed that photosynthesizing plants when transferred from light to dark , carbon dioxide production initially for 3- 4 minutes was high, than that was produced later- POST ILLUMINATION BURST OF $CO_2$.

**These were later recognized as the light dependent release of $CO_2$ due to oxygenase activity of RuBisCo enzyme and was named as photorespiration.**

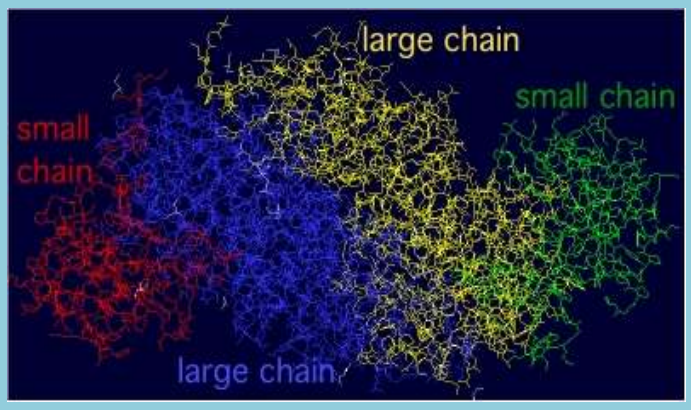
# RuBisCo ENZYME

•Ribulose-1,5-bisphosphate carboxylase-oxygenase, commonly abbreviated as RuBisCo, rubisco, RuBPCase, or RuBPco, is an enzyme involved in the first major step of carbon fixation a process by which atmospheric $CO_2$ is converted by plants and other photosynthetic organisms to energy rich molecules such as glucose.

• In plants, algae , cyanobacteria, and phototrophic and chemoautotrophic proteobacteria, the enzyme usually consists of two types of protein subunit, called the large chain (L, about 55,000 Da) and the small chain (S, about 13,000 Da).

• Large chain gene (rbcL) is encoded by chloroplastic DNA , and small chain gene by nuclear DNA(exported to chloroplast).

• The enzymatically active substrate binding site is located on the large chain, that forms dimer.




## ROLE OF RuBisCo IN PHOTORESPIRATION

- RuBisCo is a **bifunctional** enzyme- catalyzing the **carboxylation** as well as **oxidation** of substrate **RuBp**( Ribulose 1,5-bisphosphate), and thus its name.
- $CO_2$ and $O_2$ competes for reaction with RuBp ,as alternative substrates for RuBisCo, as both carboxylation & oxygenation occurs within the same active site.
- Affinity of the enzyme for $O_2$ is much lower than for $CO_2$ , still oxygenase activity is significant due to high concentration of $O_2$ than that of $CO_2$ in the chloroplast.
- Homologs of rubisco in anaerobic bacteria also exhibit oxygenase reaction, proving that this activity is intrinsically linked to the active site of the enzyme & not any environmental adaptation.
- Oxygenation of 2,3 enediol isomers RuBp yields an unstable intermediate which rabidly splits into one molecule each of **3-phosphoglycerate** & **2-phosphoglycolate**.
- 2-phosphoglycolate can't be utilized in calvin-benson cycle and is thus further metabolized by **glycolate pathway** (Edward Tolbert, 1972) also called $C_2$ cycle or oxidative photosynthetic carbon cycle.

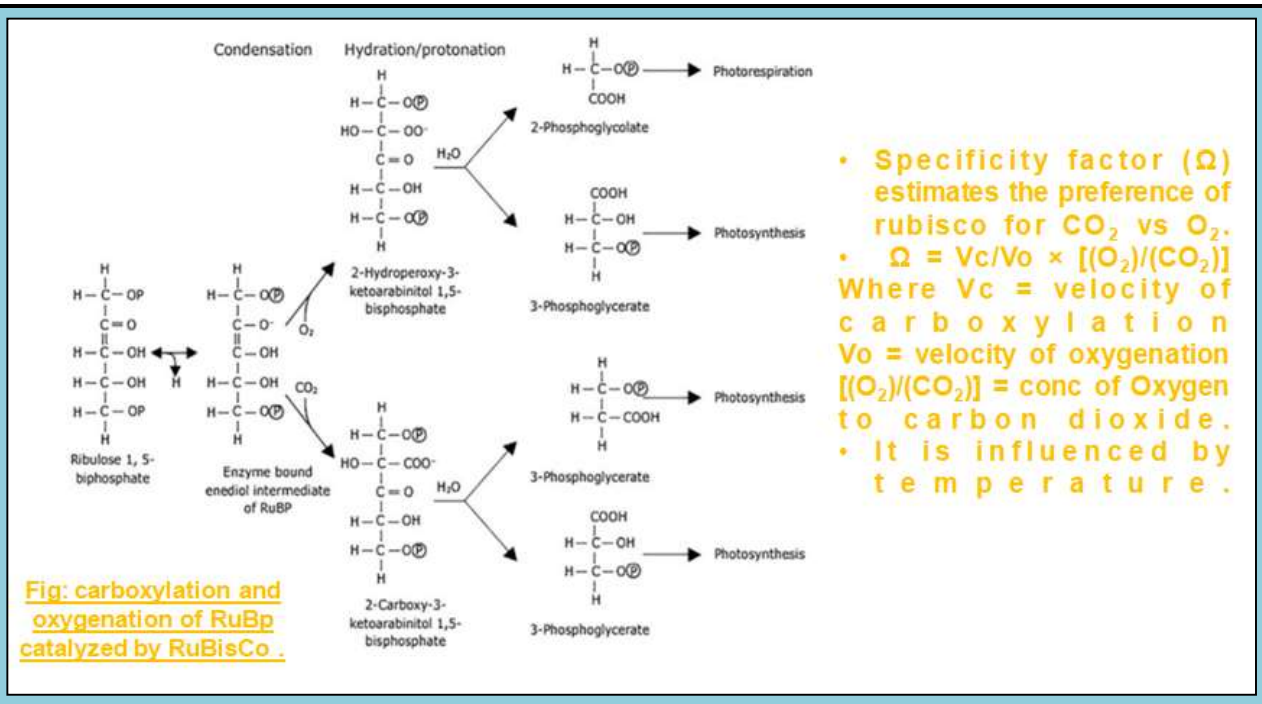



Fig: carboxylation and oxygenation of RuBp catalyzed by RuBisCo .

- Specificity factor (Ω) estimates the preference of rubisco for $CO_2$ vs $O_2$.
- Ω = Vc/Vo × $[(O_2)/(CO_2)]$

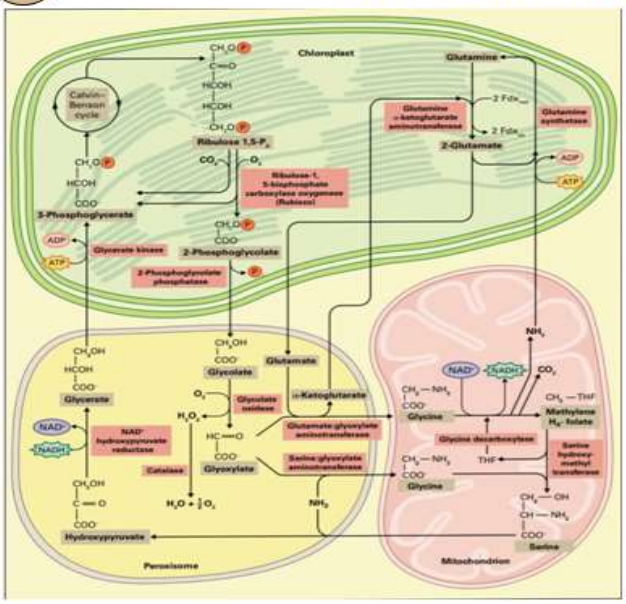
Where Vc = velocity of carboxylation
Vo = velocity of oxygenation
$[(O_2)/(CO_2)]$ = conc of Oxygen to carbon dioxide.

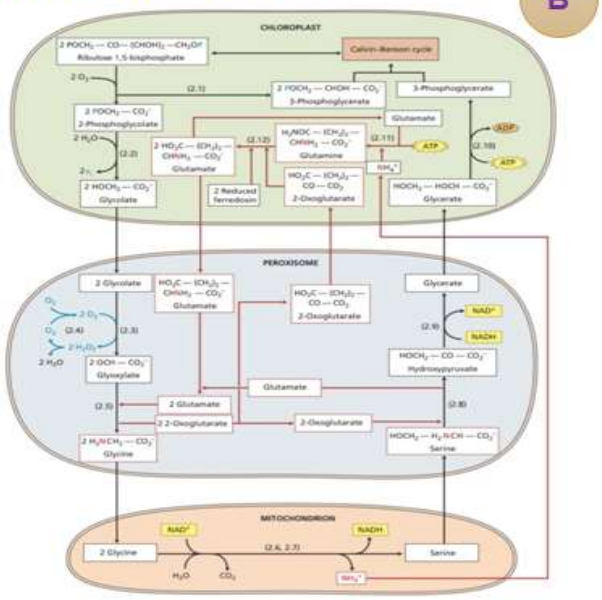
- It is influenced by temperature.

A

## PHOTORESPIRATORY PATHWAY IN PLANTS

B




A

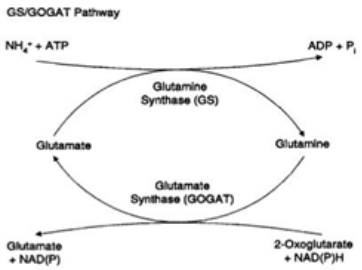
| S.I NO. | REACTION | ENZYME | SITE |
|---|---|---|---|
| 1 | 2 Ribulose 1,5-bisphosphate + 2 O2 → 2 2-phosphoglycolate + 2 3-phosphoglycerate | RuBisCO | Chloroplast |
| 2 | 2 2-Phosphoglycolate + 2 H2O → 2 glycolate + 2 Pi | Phosphoglycolate phosphatase | Chloroplast |
| 3 | 2 Glycolate + 2 O2 → 2 glyoxylate + 2 H2O | Glycolate oxidase | Peroxisome |
| 4 | 2 H2O2 → 2 H2O + O2 | Catalase | Peroxisome |
| 5 (i) | 1 Glyoxylate + 1 glutamate → 1 glycine + 1 2-oxoglutarate | Glutamate:glyoxylate aminotransferase | Peroxisome |
| 5(ii) | 1 Glyoxylate + 1 serine → 1 glycine + 1 hydroxypyruvate | Serine:glyoxylate aminotransferase | |
| 6 | 1 Glycine + NAD+ + [GDC] → CO2 + NH4+ + NADH + [GDC-THF-CH2] | Glycine decarboxylase complex (GDC) | Mitochondria |
| 7 | [GDC-THF-CH2] + 1 glycine + H2O → serine + [GDC] | Serine hydroxymethyl transferase | Mitochondria |
| 8 | Serine → hydroxypyruvate | Serine:glyoxylate aminotransferase | Peroxisome |
| 9 | Hydroxypyruvate + NADH + H+ → glycerate + NAD+ | Hydroxypyruvate reductase | Peroxisome |
| 10 | Glycerate + ATP → 3-phosphoglycerate + ADP | Glycerate kinase | Chloroplast |
| 11 | Glutamate + NH4+ + ATP → glutamine + ADP + Pi | Glutamine synthetase | Chloroplast |
| 12 | 2-Oxoglutarate + glutamine + 2 Fd(red) + 2 H+ → 2 glutamate + 2 Fd(oxid) | Ferredoxin-dependent glutamate synthase (GOGAT) | Chloroplast |

B

| Reaction* | Enzyme |
|---|---|
| 1. 2 Ribulose 1,5-bisphosphate + 2 $O_2$ → 2 2-phosphoglycolate + 2 3-phosphoglycerate | Rubisco |
| 2. 2 2-Phosphoglycolate + 2 $H_2O$ → 2 glycolate + 2 $P_i$ | Phosphoglycolate phosphatase |
| 3. 2 Glycolate + 2 $O_2$ → 2 glyoxylate + 2 $H_2O_2$ | Glycolate oxidase |
| 4. 2 $H_2O_2$ → 2 $H_2O$ + $O_2$ | Catalase |
| 5. 2 Glyoxylate + 2 glutamate → 2 glycine + 2 2-oxoglutarate | Glutamate:glyoxylate aminotransferase |
| 6. Glycine + $NAD^+$ + [GDC] → $CO_2$ + $NH_4^+$ + NADH + [GDC-THF-$CH_2$] | Glycine decarboxylase complex (GDC) |
| 7. [GDC-THF-$CH_2$] + glycine + $H_2O$ → serine + [GDC] | Serine hydroxymethyl transferase |
| 8. Serine + 2-oxoglutarate → hydroxypyruvate + glutamate | Serine:2-oxoglutarate aminotransferase |
| 9. Hydroxypyruvate + NADH + $H^+$ → glycerate + $NAD^+$ | Hydroxypyruvate reductase |
| 10. Glycerate + ATP → 3-phosphoglycerate + ADP | Glycerate kinase |
| 11. Glutamate + $NH_4^+$ + ATP → glutamine + ADP + Pi | Glutamine synthetase |
| 12. 2-Oxoglutarate + glutamine + 2 $Fd_{red}$ + 2 $H^+$ → 2 glutamate + 2 $Fd_{ox}$ | Ferredoxin-dependent glutamate synthase (GOGAT) |

## THE PCO CYCLE IS INTERCONNECTED WITH MANY METABOLIC PATHWAYS.

1. **NITROGEN METABOLISM:** The $NH4^+$ released in the oxidation of glycine diffuses from the mitochondrial matrix to the chloroplasts, where glutamine synthetase catalyzes the ATP-dependent incorporation of $NH4^+$ into glutamate, yielding glutamine, ADP, and inorganic phosphate. Glutamine and 2-oxoglutarate are substrates of ferredoxin-dependent glutamate synthase (GOGAT) for the production of two molecules of glutamate, restored by reassimilation of NH4+, for peroxisomal aminotransferase activity.



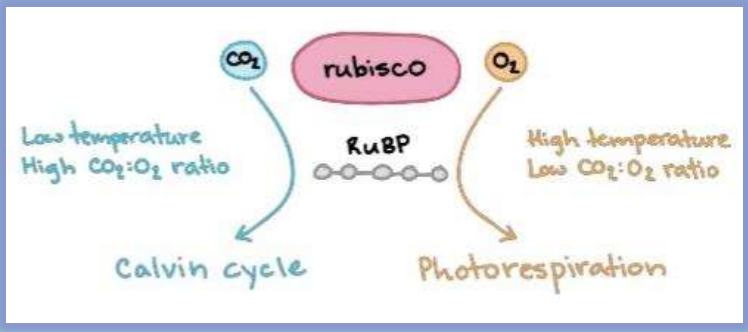

**2. ELECTRON TRANSPORT CHAIN:** ATP for the transformation of glycerate to 3-phosphoglycerate , comes from ETC. It also provides $NAD^+$, for glycine decarboxylase activity and 2 molecules of reduced ferredoxin for incorporating $NH4^+$ into glutamate via glutamine synthase & salvaging glutamate by GOGAT enzyme.

**3.CELL REDOX HOMEOSTASIS:** $H_2O_2$ damages key cellular molecules such as DNA and lipids, thus is broken down to $H_20$ & $O_2$ by catalase. However it also acts as a signal molecule between hormones and stress response.

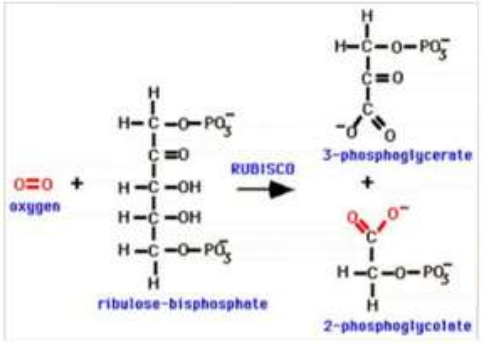
**4. C1 METABOLISM:** 5, 10-methylene tetrahydrofolate is the cofactor required by glycine decarboxylase–serine hydroxymethyltransferase in the conversion of glycine to serine in the mitochondria. Reactions mediated by folates transfer one-carbon units in the synthesis of precursors for proteins, nucleic acids, lignin, and alkaloids.

# FACTORS INFLUENCING PHOTORESPIRATION

- Kinetic property of Rubisco: as $K_m \propto 1/v_0$ , when Km of $CO_2$ is high velocity of carboxylation decreases because Rubisco chooses $O_2$ over it.
- Temperature : high temperature promotes oxygenation of RuBp, as solubility of $CO_2$ in water declines more rapidly than that of $O_2$. And specificity factor of Rubisco also decreases.
- Concentration of substrates $CO_2$ and $O_2$ : On a hot bright day, when photosynthesis has depleted $CO_2$ in the chloroplasts and raised the level of $O_2$, the rate of photorespiration increases , as conc ratio of $CO_2$ to $O_2$ decreases.



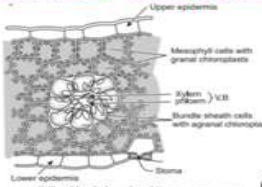

## USEFUL OR WASTEFUL



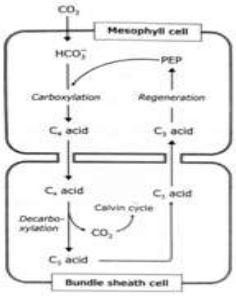

- The oxygenase activity of rubisco causes partial loss of the carbon fixed by the Calvin–Benson cycle and yields **2-phosphoglycolate**, an **inhibitor** of two chloroplast enzymes: **triose phosphate isomerase** and **phosphofructokinase**. To avoid both drain of carbon out of the Calvin–Benson cycle and enzyme inhibition, 2-phosphoglycolate is metabolized through the C2 oxidative photosynthetic carbon cycle back to **3-phosphoglycerate** a stable intermediate of Calvin Benson cycle.
- Release of $CO_2$ , with the utilization of ATP, decreases photosynthetic output – **Drain on photosynthetic carbon fixation (Lawlor,1987)**.
- 2 molecules of 2-phosphoglycolate(4 carbon) converted to 1 3-PG(3carbon) & 1 $CO_2$ (1 carbon) results In **recovery of 75% of carbon** lost due to oxygenation of RuBp.

## WHY PHOTORESPIRATION IS LOW IN C4 PLANTS BUT HIGH IN C3 PLANTS

- **C3 plants**: In the presence of light , $O_2$ level increases in the chloroplast , promoting oxygenase activity of RuBisCo, where $O_2$ successfully competes over $CO_2$ for the binding site of the enzyme.
- **C4 plants:** characterized by **Kranz's anatomy** & presence of '**$CO_2$ enrichment pathway**', $CO_2$ from external atmosphere moves to the mesophyll cells , & then to the chloroplasts in bundle sheath cells , concentrating $CO_2$ there. Thus the ratio of $CO_2$:$O_2$ increases in bundle sheath cells than the external atmosphere, permitting $CO_2$ to compete effectively with $O_2$ for carboxylase activity.



T.S. of leaf showing Kranz anatomy



**C4 CYCLE**

## C3 PLANTS PERFORMING PHOTORESPIRATION


Wheat


Soybean


Alfalfa


Tobacco

## SIGNIFICANCE

- Carbon salvaging mechanism
- Protection from photo-inhibition
- Removes toxic metabolic intermediate
- Integrated Into primary Metabolism

# Conclusion

And the end to come to know that photorespiration being a necessary part of Photosynthesis not only links oxygen fixation with the Calvin cycle but also aids several metabolic processes. The unique role of

RuBisCo here portrays the dual nature of this enzyme. The C3 plants are specialized to carry out this process in case the concentration of oxygen in the chloroplast near the rubisco enzyme increases. It may appear to drain out Carbon aimed to be fixed by the Calvin cycle, but ultimately proves to be significant as well.

# Acknowledgement

I would like to express my gratitude towards Dr.Tripti Roy and Dr. Debjani Sinha Ray, Associate Professors of the department of Botany, Bethune college, Kolkata, for providing me this opportunity, for expressing myself through my topic. Thank you ma'am for your support and guidance.

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# Paper-VII

# Z-scheme Of Photosynthesis

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# Abstract

The “Z-scheme” describes the oxidation/reduction changes during the light reactions of photosynthesis. The vertical axis in the figure represents the reduction potential of a particular species—the higher the position of a molecular species, the more negative its reduction potential, and the more easily it donates electrons. In the Z-scheme, electrons are removed from water (to the left) and then donated to the lower (non-excited) oxidized form of P680. Absorption of a photon excites P680 to P680*, which “jumps” to a more actively reducing species. P680* donates its electron to the quinone-cytochrome bf chain, with proton pumping. The electron from cytochrome bf is donated to PSI, converting P700 to P700*. This electron, along with others, is transferred to NADP, forming NADPH. Alternatively, this electron can go back to cytochrome bf in cyclic electron flow.

# Introduction

▶ Photosynthesis occur in two step :

1. Light dependent reaction (light reaction)
2. Light independent reaction(dark reaction)

▶ Light reaction in the photo-system starts electron flow which is known as Z-scheme.

▶ The “**Z-scheme**” describes the oxidation/reduction changes during the light reactions of photosynthesis.

▶ Light-Dependent Reactions (**Z Scheme**) happen in the thylakoid membrane of the chloroplasts and **occur** in the presence of sunlight.

▶ In green plants ,flow of electron is of two type

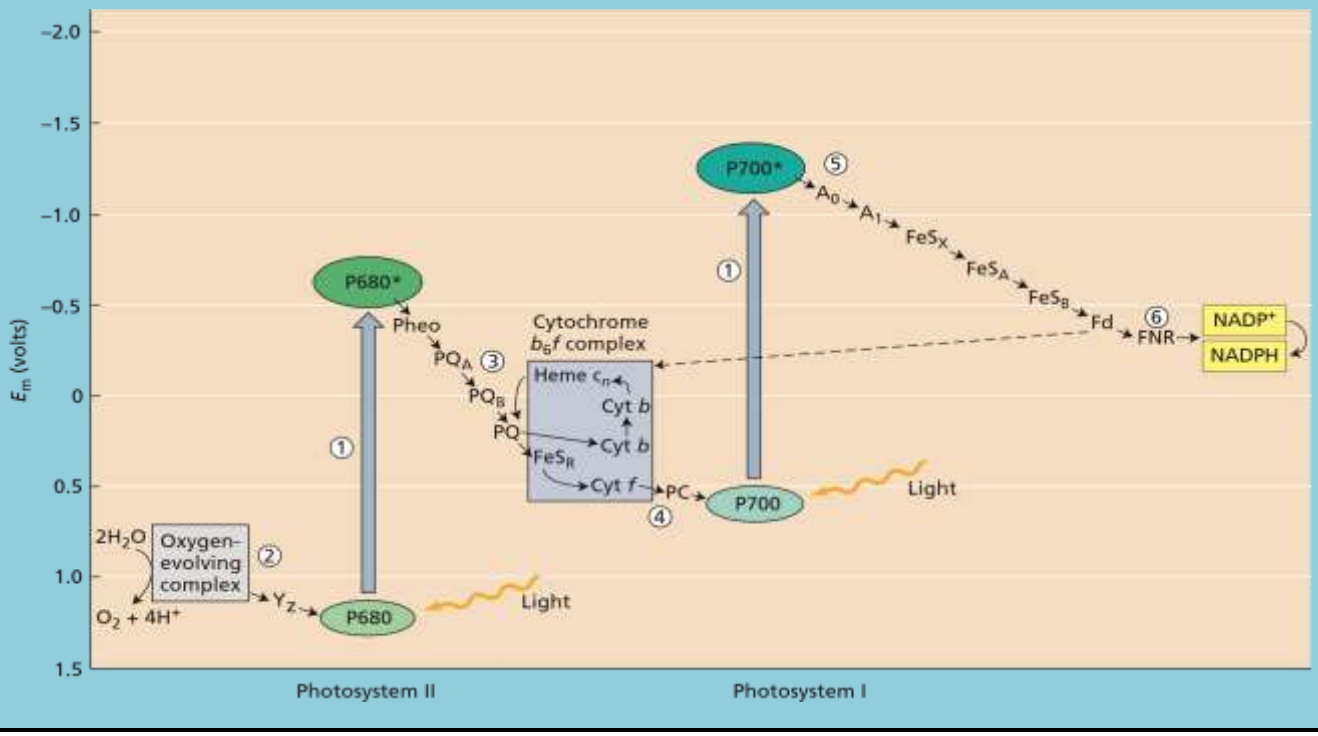
a)non-cyclic b) cyclic

# NONCYCLIC ELECTRON FLOW:

It is a light-induced electron transport form water to NADP+ and a concomitant evolution of oxygen . It involves a collaboration of two photo-system: PSII and PSI electrons move from water through PSII to PSI and then $NADP^+$. Electron transport leads to generate of a proton-motive force and synthesis of ATP. Formation of ATP due to light-induced non-cyclic electron flow is called non-cyclic photo-phosphorylation.

# CYCLIC ELECTRON FLOW:

In certain cases ,photo-excited electrons follow cyclic path , which involves PSI and PSII. In cyclic electron flow, photo-excited electrons from P700 of PSI move through the b6f complex and back to P700.The cyclic electron flow is coupled to proton pumping into the thylakoid lumen .When protons flow down their electrochemical gradient through ATP synthase complexes, ATP synthesis occurs. The formation of ATP due to light-induced cyclic electron flow is cyclic photophosphorylation.



# PATHWAY

(1) The vertical arrows represent photon absorption by the reaction center chlorophylls:P680 for photosystem II (PSII) and P700 for photosystem I (PSI). The excited PSII reaction center chlorophyll, P680*, transfers an electron to pheophytin ( Pheo ).

(2) On the oxidizing side of PSII (to the left of the arrow joining P680 with P680*), P680 oxidized by light is re-reduced by Yz , which has received electrons from oxidation of water.

(3)On the reducing side of PSII (to the right of the arrow joining P680 with P680*), pheophytin transfers electrons to the acceptors PQA and PQB, which are plastoquinones .

(4) The cytochrome *b*6*f* complex transfers electrons to plastocyanin(PC), a soluble protein, which in turn reduces P700+ (oxidized P700).

(5) The acceptor of electrons from P700* (A0) is thought to be a chlorophyll, and the next acceptor (A1) is a quinone. A series of membrane-bound iron–sulfur proteins(FeSX, FeSA, and FeS) transfers electrons to soluble ferredoxin (Fd).

(6) The soluble flavoprotein ferredoxin–NADP+ reductase (FNR) reduces NADP+ to NADPH, which is used in the Calvin–Benson cycle to reduce CO2 .The dashed line indicates cyclic electron flow around PSI.

# Q cycle

## Mechanism of electron and proton transfer in the cytochrome *b6f* complex.

•This complex contains two *b*-type cytochromes (Cyt *b*), a *c*-type cytochrome (Cyt *c*, historically called cytochrome *f* ), a Rieske Fe–S protein (FeSR), and two quinone oxidation–reduction sites.

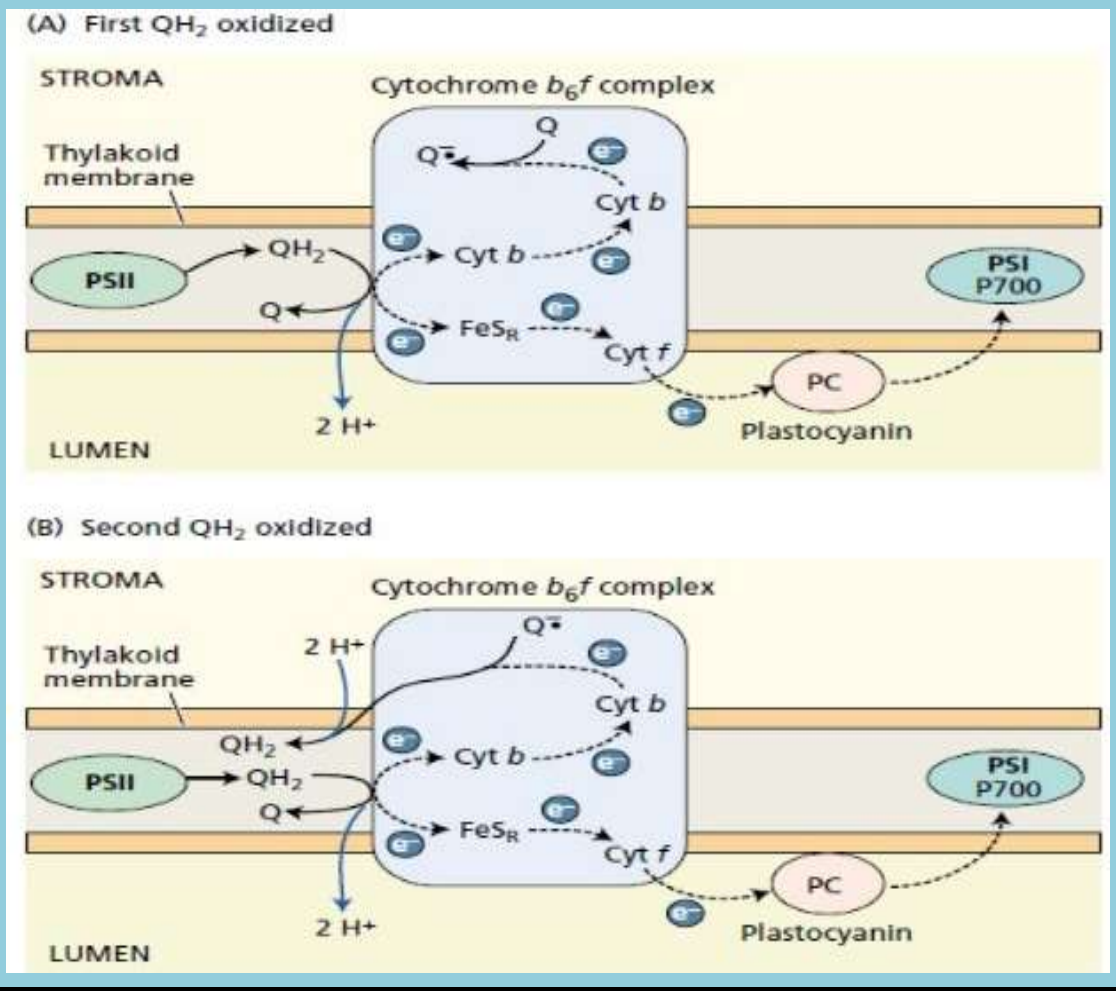
•(A) The noncyclic or linear processes: A plastohydroquinone (PQH2) molecule produced by the action of PSII is oxidized near the lumenal side of the complex, transferring its two electrons to the Rieske Fe–S protein and one of the *b*-type cytochromes and simultaneously expelling two protons to the lumen.

•The electron transferred to FeSR is passed to cytochrome *f* (Cyt *f* ) and then to plastocyanin (PC), which reduces P700 of PSI.

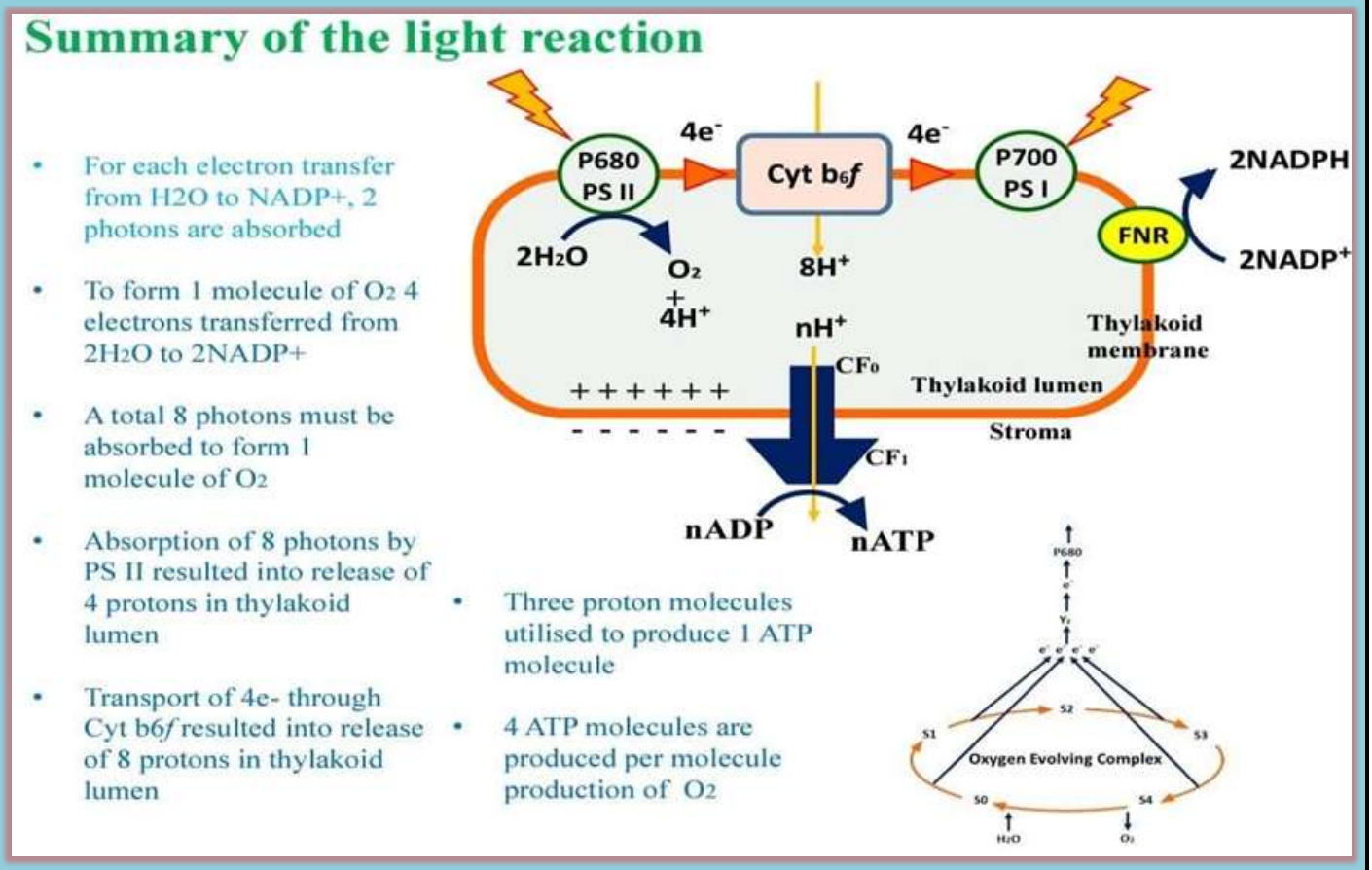
•The reduced *b*-type cytochrome transfers an electron to the other *b*-type cytochrome,which reduces a plastoquinone (PQ) to the plastosemiquinone (PQ•– ) state.

•The cyclic processes: A second PQH2 is oxidized, with one electron going from FeSR to PC and finally to P700.

•The second electron goes through the two *b*-type cytochromes and reduces the plastosemiquinone to the plastohydroquinone,at the same time picking up two protons from the stroma.



▶ Overall, four protons are transported across the membrane for every two electrons delivered to P700.




# Conclusion

The Z-scheme is an energy diagram for electron transfer in the "light reactions" of plant photosynthesis. It applies equally well to photosynthesis by algae and cyanobacteria. The vertical energy scale shows each molecule's ability to transfer an electron to (i.e., to reduce) the next one from left to right

# Acknowledgement

I would like to express my gratitude to Dr.Tripti Roy and Dr.Debjani Sinha Roy, associate professors of Department of Botany, Bethune College , for giving me this opportunity to present this topic. I also like to thank my all classmates.

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Paper-VIII

# PHLOEM SAP AND P-PROTEIN

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# Abstract

Phloem sap consist primarily of sugars, hormones, and mineral elements dissolved in water. It flows from where carbohydrates are produced or stored to where they are used. Other mobile carbohydrates such as Raffinose, Stachyose and Verbascose are also translocated with carbohydrates(sucrose). Translocated sugar alcohol includes mannitol and sorbitol. Nitrogen found in phloem sap is largely amino acids, specially glutamate and aspartate or aspartic acid and their amides glutamine and asparagine. A variety of proteins and RNAs found in phloem. Almost all of the endogenous plant harmones found in phloem tissue. Protein present in phloem sap include structural protein like $PP_1$ & $PP_2$ as well as a no. of water soluble protein. The main phloem protein involved in sealing damaged seive elements are the structural protein called P-protein. It was previously known as slime bodies. It occurs in different forms such as tubular, fibrillar, granular crystalline, etc. In immature cells P-proteins occur as discrete bodies in the cytosol, known as P-protein bodies. It may be spherical, spindle shaped or twisted and coiled. P-protein have been characterized at the molecular level. P-protein function in sealing of damaged seive elements by plugging up the seive plate pores. Protein crystals release from ruptured plastids also play a similar role in case of some monocotyledons. P-protein also play a role in defence mechanism if seive tubes are attached by sap sucking invaders like insects and causing rupture to seive.

# Introduction

The phloem is the major route for the translocation and distribution of organic metabolites assimilated during photosynthesis. The sieve elements (SEs) transport a wide range of compounds like water, minerals, amino acids, organic acids, sugars, and sugar alcohols. Research in recent years has shown that the phloem system is not only responsible for photosynthate allocation, but has several additional functions. For example, the phloem is an important mediator of whole-plant communication (Ruiz-Medrano et al., 2001). The transported information molecules include phytohormones (Baker, 2000), and also macromolecules such as proteins (Pearce, 1991) and RNAs (Jorgensen et al., 1998; Jorgensen, 2002). The occurrence of macromolecules seems surprising, since mature SEs lack the capability for mRNA and protein synthesis. However, recent studies have provided accumulating evidence that these macromolecules only not sporadically appear, but a large number of RNAs (Lucas et al., 2001) and soluble proteins (Hayashi et al., 2000; Walz et al., 2004) are constantly present in SE exudate. Proteins can even be regarded as a major component of phloem sap, given that, for example, cucurbit exudate contains high concentrations up to 100 mg ml−1 (Richardson et al., 1982). Phloem sap proteins are believed to be imported through specialized plasmodesmata connecting SEs and the adjacent companion cells (CCs) where protein synthesis is taking place.

# PHLOEM SAP

•Phloem sap consist primarily of sugars, hormones, and mineral elements dissolved in water.

•It flows from where carbohydrates are produced or stored to where they are used.

•Other mobile carbohydrates such as Raffinose, Stachyose and Verbascose are also translocated with carbohydrates(sucrose).

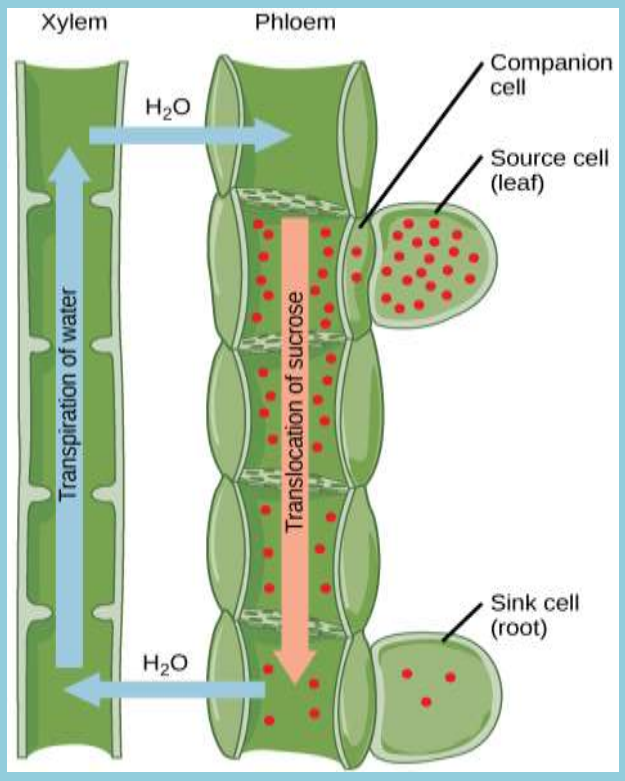
•Translocated sugar alcohol includes mannitol and sorbitol.

•Nitrogen found in phloem sap is largely amino acids, specially glutamate and aspartate or aspartic acid and their amides glutamine and asparagine.

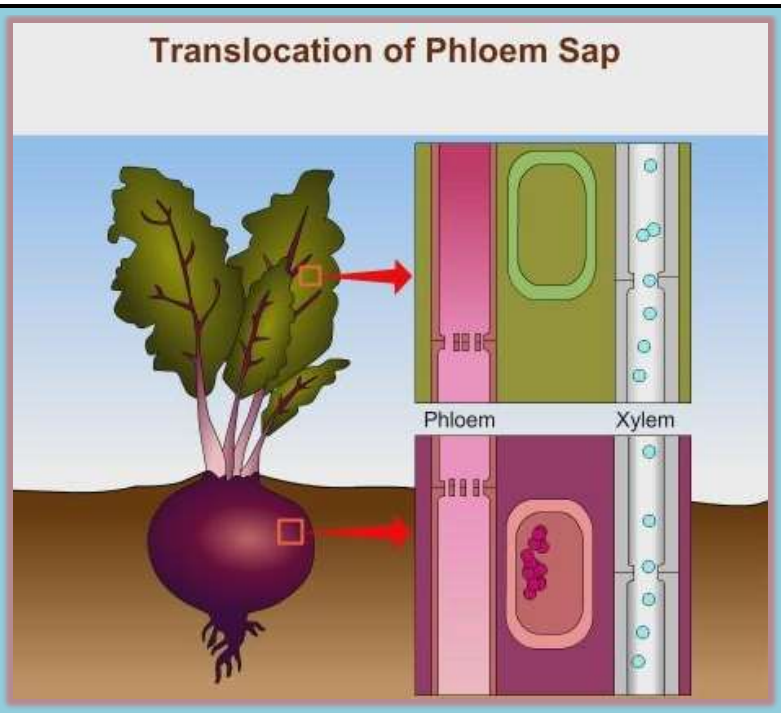
•A variety of proteins and RNAs found in phloem.

•Almost all of the endogenous plant harmones found in phloem tissue.

•Protein present in phloem sap include structural protein like $PP_1$ & $PP_2$ as well as a no. of water soluble protein.




**Phloem Transport**




# P-PROTEIN

•The main phloem protein involved in sealing damaged seive elements are the structural protein called P-protein.

•It was previously known as slime bodies.

•It occurs in different forms such as tubular, fibrillar, granular crystalline,etc.

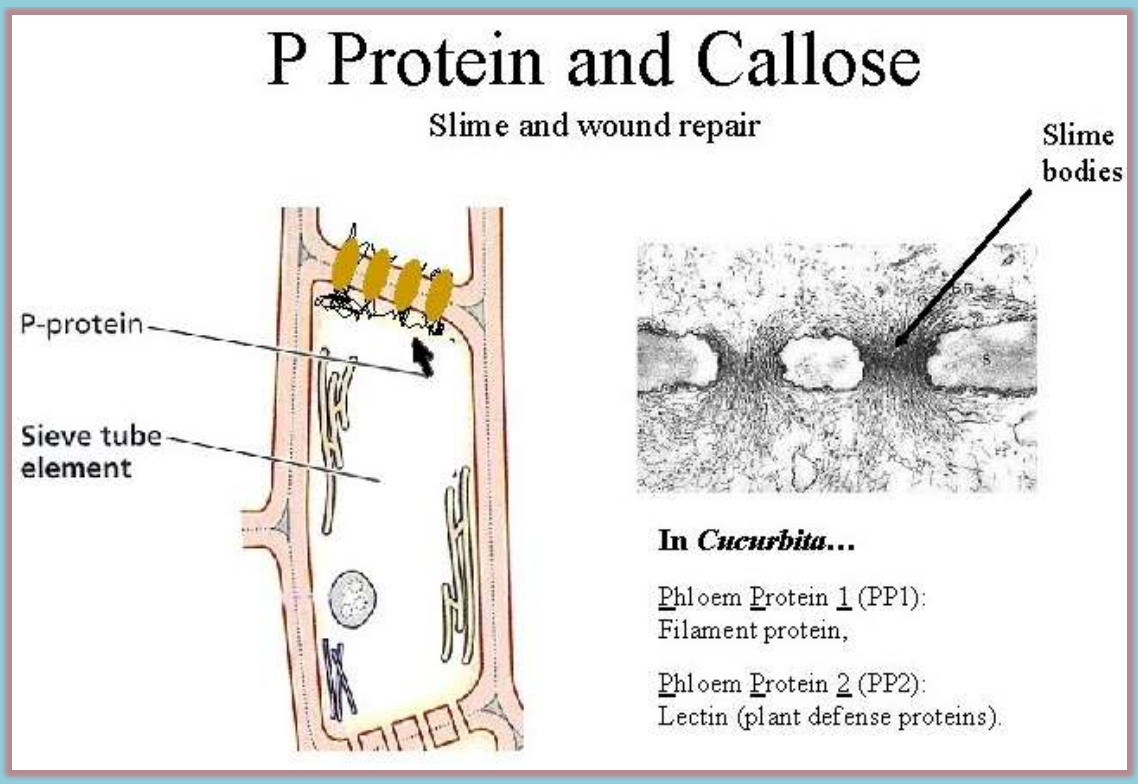
•In immature cells P-proteins occur as discrete bodies in the cytosol, known as P-protein bodies.

•It may be spherical, spindle shaped or twisted and coiled.

•P-protein have been characterized at the molecular level.

# FUNCTIONS OF P-PROTEIN

- P-protein function in sealing of damaged seive elements by plugging up the seive plate pores.
- Protein crystals release from ruptured plastids also play a similar role in case of some monocotyledons.
- P-protein also play a role in defence mechanism if seive tubes are attached by sap sucking invaders like insects and causing rupture to seive.




# Conclusion

The recent identification of a growing number of proteins from phloem sap of different plant species now allows first insights into the potential functions of these polypeptides. Functional classification maps them to diverse categories but, interestingly, a number of them are functionally related to defence responses and therefore influences on plant–insect interactions are conceivable.

The most likely functions are connected to instant wound signalling, plugging of SEs to avoid nutrient loss, and, since there is evidence that herbivores as well as phloem feeders are able to take up and digest phloem sap proteins, the dispersal of directly acting anti-insect polypeptides.

Future studies analysing the direct effects of insect infestation on local and systemic changes of phloem sap protein composition and activity will elucidate their exact involvement in plant defence against herbivores and phloem-sucking insects. This knowledge will be useful to develop novel biotechnological strategies to enhance the resistance of crop plants against phloem-feeding insects.

## Acknowledgement

I would like to extend my gratitude towards my teachers Dr. Tripti Roy and Dr. Debjani Sinha Roy for providing me this opportunity of giving a presentation on this topic. I would also like to thank all my departmental teachers for teaching me and thus making me well informed about this particular topic. Last but not the least I would like to thank all my fellow classmates for bearing with me in this entire period of time.

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# Paper-IX

# CALVIN CYCLE

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# Abstract

The Calvin cycle is a reductive process in the stroma of chloroplasts responsible for the synthesis of carbohydrates from carbon dioxide. The reactions are organized in a cyclic metabolic pathway that was named after its discoverer Melvin Calvin who received the Nobel Price for Chemistry in 1961. The reactions of Calvin Cycle can be organized into three basic stages:-1. Fixation or Carboxylation 2. Reduction 3. Regeneration. Reducing power in the form of NADPH (nicotinamide-adenine dinucleotide phosphate reduced form) and energy as adenosine triphosphate (ATP) required for this process are generated in the light reactions located in the thylakoid membrane. Light activation of this process is achieved by covalent redox-modification of some key enzymes that are inactive in the dark. The other names of Calvin cycle are C3 Cycle, light independent pathway, reductive pentose phosphate reaction, photosynthetic carbon reduction cycle, etc.

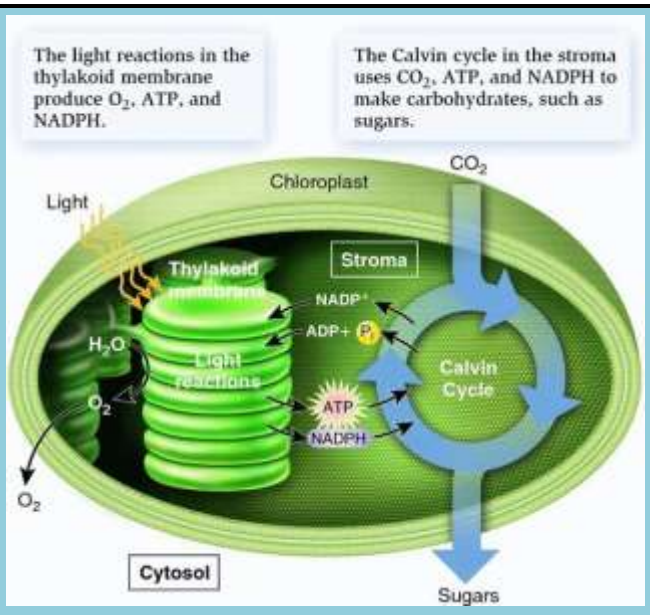
# Introduction

Photosynthesis is divided into two primary phases:-

1. Light reaction
2. Dark reaction also known as Calvin cycle.

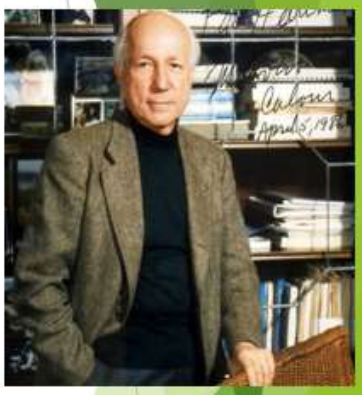
What is Calvin cycle ?

Calvin cycle is a cyclic reaction occuring in the dark phase of photosynthesis in which Carbondioxide($CO_2$) is converted into glucose which is then utilized by the plants.




# Discovery of Calvin cycle

- The Calvin cycle was first observed by Melvin Calvin In 1950 on chlorella,unicellular green algae.
- Calvin was awarded with Nobel prize for this work in 1961.
- Calvin and his co-worker while working at the University of Berkelly,he used a Carbon-14 isotopes to understand the photosynthesis process in plants.
- This radioactive isotope helped him to determine how light independent reaction works in single-celled algae.
- Calvin and his co-worker after starting the experiment found that after 3 seconds the radioactive isotope was found in phosphoglycerate.
- Phosphoglycerate is, therefore, the first stable product of Calvin cycle.



# OTHER NAMES OF CALVIN CYCLE ARE:-

- C3 Cycle
- Dark reaction
- Light-independent reaction
- Reductive Pentose phosphate reaction
- Photosynthetic carbon reduction cycle

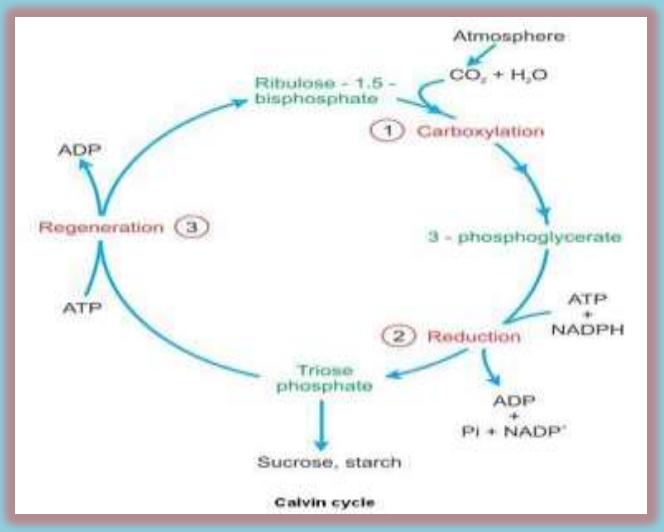
# STAGES OF C3 CYCLE:

▶ The Calvin cycle takes place in the stroma of the chloroplast,the energy required in the whole pathway are also found in stroma of chroloplast.

▶ The Calvin cycle is not totally independent of light since it relies on ATP and NADPH,which are the product of light dependent reactions.

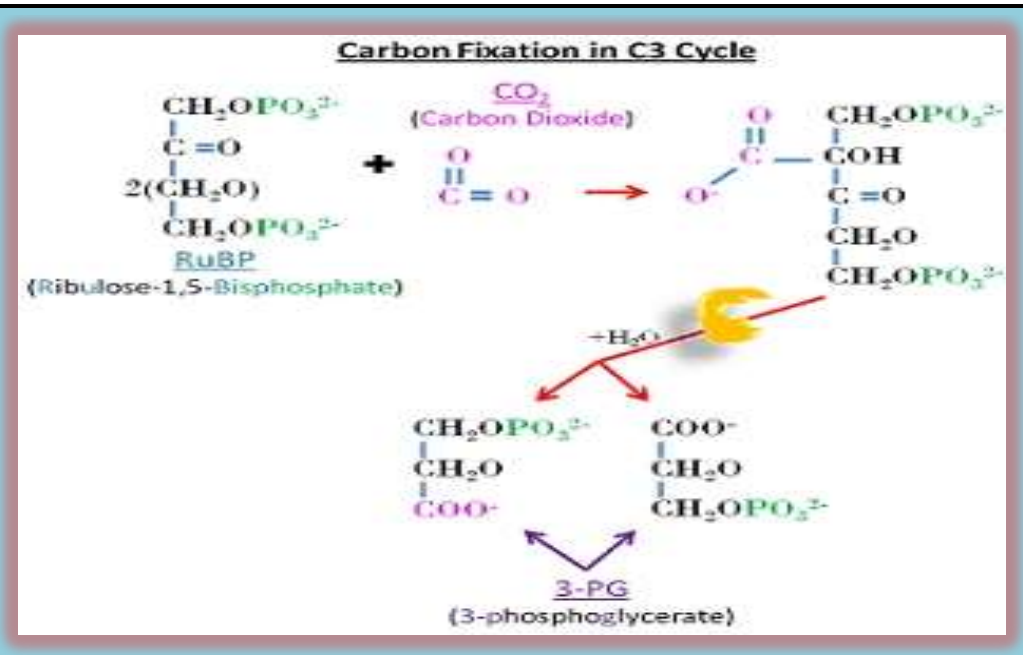
▶ The reactions of Calvin Cycle can be organized into three basic stages:-

- **Fixation or Carboxylation**
- **Reduction**
- **Regeneration**



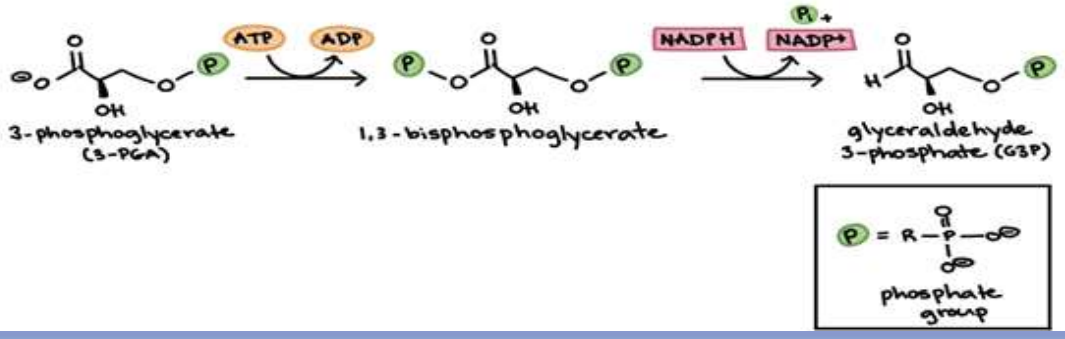

## Fixation

- In the stroma,in addition to CO2 ,two other components are present to initiate the light-independent reactions:- an enzyme called ribulose Bisphosphate carboxylase(RUBISCO)and three molecules of ribulose bisphosphate(RUBP).
- RUBP has five carbons,flanked by two phosphates.
- For each CO2 molecules that reacts with one RUBP,producing an unstable intermediate called 2-carboxy 3-keto 1,5-biphosphoribotol which immediately splits into two molecules of 3 phosphoglycerate (3PGA),it has 3 carbons and 1 phosphate.
- In calvin cycle ,fixation occurs 3 times producing 6 molecules of 3PGA.



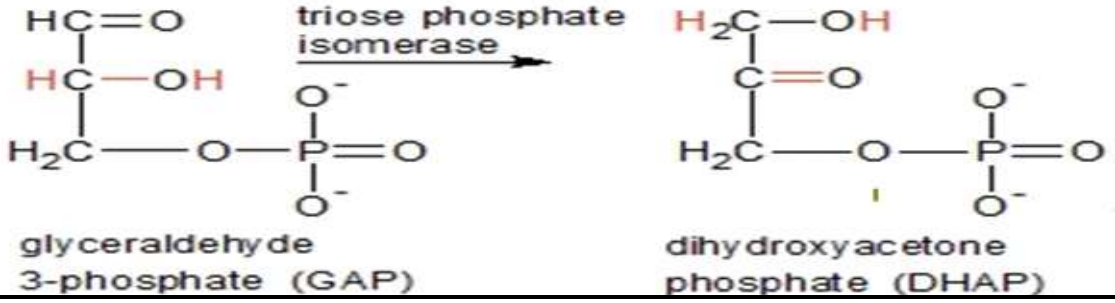

# Reduction

- 6 molecules of 3PGA are phosphorylated to form 6 molecules of 1,3 bisphosphoglycerate and 6 molecules of ADP in the presence of enzyme 3-phosphoglycerate kinase.
- 6 molecules of 1,3 bisphophoglycerate are reduced by using 6 molecules of NADPH in the presence of enzyme glyceraldehyde-3-phosphate dehydrogenase to form 6 molecules of Glyceraldehyde 3-phosphate(G3P),6 NADP and phosphate group.




# Regeneration

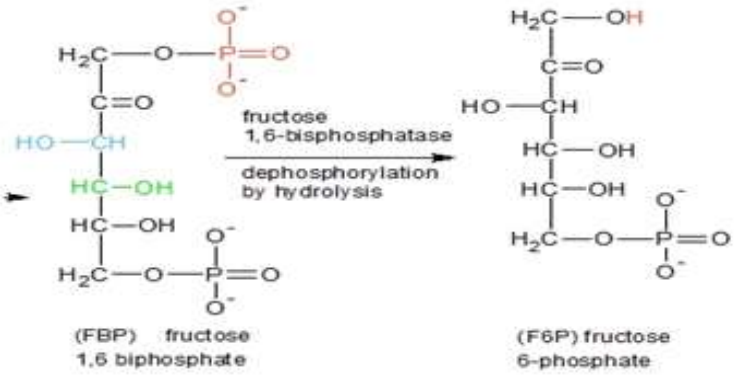
- In the Regeneration phase,the ribulose -1,5 bisphosphate is regenerated ,it involves the following steps:-
  - Some molecules of glyceraldehyde 3-phosphate(G3P) is converted into dihydroxyacetone 3-phosphate (DHAP) in the presence of enzyme Triose phosphate isomerase.



- Both G3P and DHAP unite in the presence of enzyme, aldolase to form fructose 1,6-bisphosphate.

3 Phospho glyceraldehyde + DHAP —Aldolase→ Fructose 1,6 diphosphate

- Fructose 1,6-bisphosphate is Converted into fructose -6-phosphate in the presence of enzyme fructose 1,6 bisphosphatase.




- Glyceraldehyde-3-phosphate reacts with fructose-6-phosphate in the presence of Transketolase as enzyme forms erythrose-4-phosphate and xylulose-5-phosphate.

3 Phospho glyceraldehyde + Fructose 6 phosphate —Trans ketolase→ Erythrose 4 phosphate + Xylulose 5 phosphate

- Erythrose-4-phosphate react with DHAP In presence of enzyme aldolase to form sedoheptulose 1,7-bisphosphate.

Erythrose 4 phosphate + DHAP —Aldolase→ Sedoheptulose 1,7 diphosphate

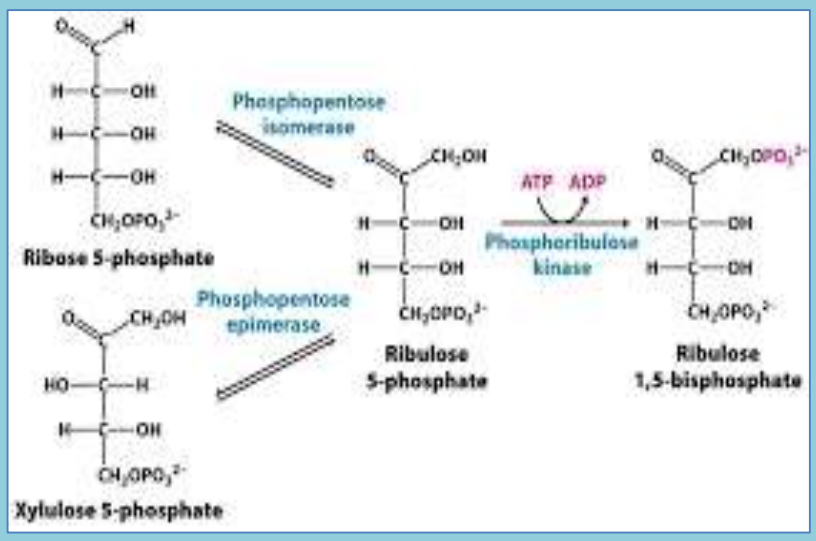
- Sedoheptulose 1,7-bisphosphate loses one phosphate group in presence of enzyme sedoheptulose 1,7-bisphosphatase to form sedoheptulose-7-phosphate.
- Sedoheptulose-7-phosphate reacts with glyceraldehyde-3-phosphate in the presence of enzyme Transketolase to form xylulose-5-phosphate and ribose-5-phosphate.

Sedoheptulose 7 phosphate + 3 Phospho Glyceraldehyde —Trans Ketolase→ Xylulose 5 phosphate + Ribose 5 phosphate

▶ Ribose-5-phosphate is converted into ribulose-5-phosphate in presence of enzyme phosphopentose isomerase.

▶ Xylulose-5-phosphate is then converted into ribulose-5-phosphate in the presence of enzyme phosphopentose epimerase.

▶ Ribulose-5-phosphate is phosphorylated by ATP in the presence of phosphoribulokinase to form Ribulose 1,5-bisphosphate and ADP.



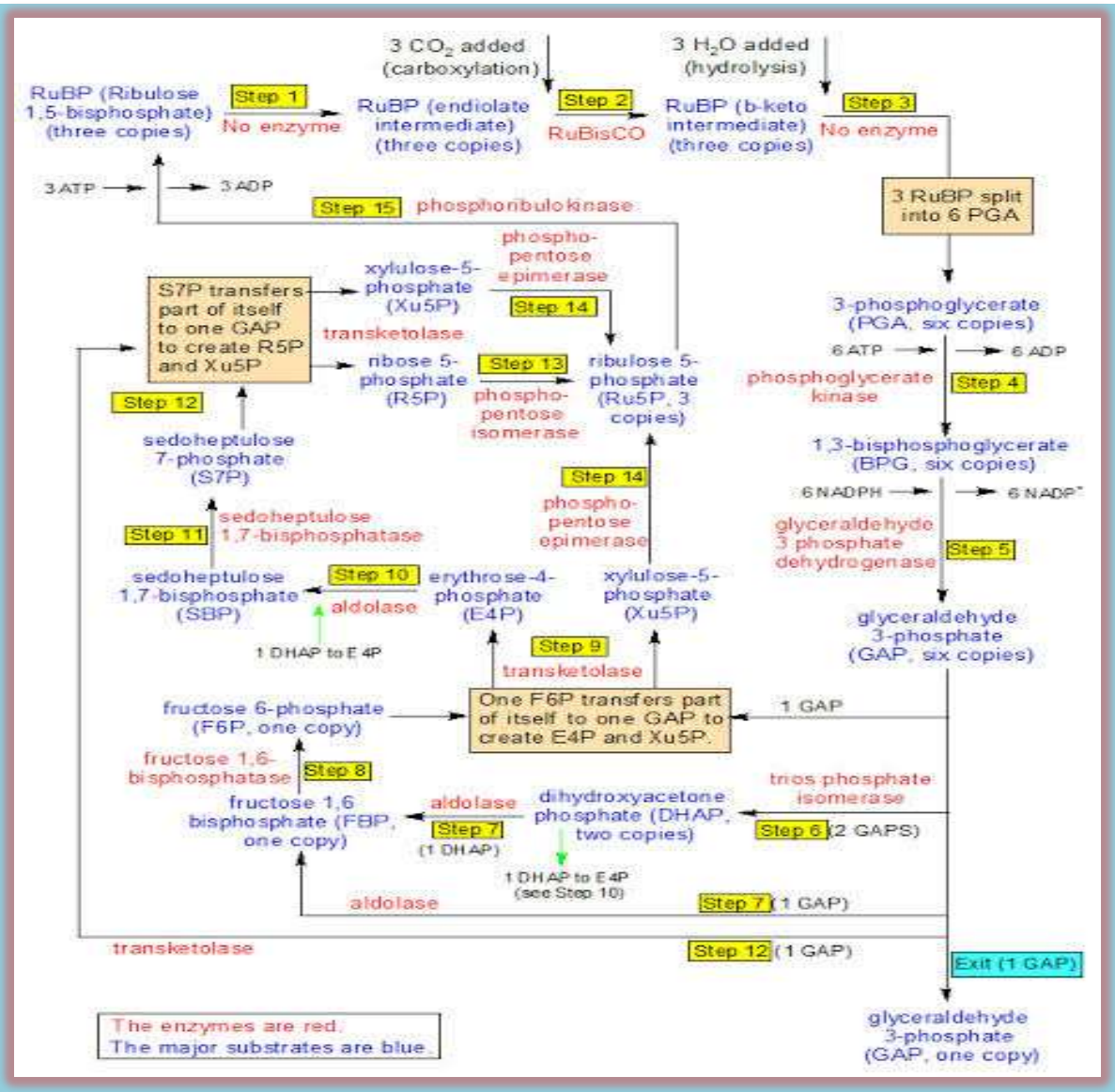

## Net reaction : —

$3CO_2 + 5H_2O + 6NADPH + 9ATP \rightarrow$ glyceraldehyde - 3 - phosphate + $6NADP^+$ + $3H^+$ + 9 ADP + 8Pi

## Significance of Calvin Cycle

- It is the main biochemical pathway during the dark reaction(phase II) of photosynthesis.
- It results in the synthesis of carbohydrates from $CO_2$(assimilation of carbon).
- It depends on the light reaction(phase I) for the supply of assimitory energy(ATP and $NADPH_2$) required for carbon assimilation.

- It stores the ATP energy formed during light reaction in the carbohydrate molecules as the food energy.
- It is the primary source of organic food and food energy for all the organisms.
- Calvin cycle(C3 Cycle) occurs in all photosynthetic plants i.e.C3,C4,CAM plants during the dark phase of photosynthesis.




# Conclusion

So, the Calvin cycle, light-independentreactions, bio synthetic phase, dark reactions, or photosynthetic carbon reduction (PCR) cycle of photosynthesis are the chemical reactions that convert carbon dioxide

and other compounds into glucose using the reducing power ATP and NADPH from the light dependent reactions.

# Acknowledgement

I would to express my gratitude to Dr.Tripti Roy and Dr.Debjani Sinha Roy for giving me this opportunity to speak and present my topic and I would like to thank them for their constant support. I would also like to thank all my friends for their patience during my presentation.

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# Paper-X

# VERNALIZATION: PROMOTING FLOWERING WITH COLD

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# Abstract

Besides an appropriate photoperiod, certain plants require a low temperature treatment during their earlier stages of the life history for subsequent flowering in the later stages. The conversion of the winter wheat into the spring variety after slight germination by low temperature or chilling treatment was later coined as vernalization by a Russian geneticist, Triofim Denisovich Lysenko (1928). The mechanism of vernalization is obscure. This article summarizes two major hypothetical theories – Phasic Development Theory (by Lysenko, 1934) and Hormonal Hypothesis (by Lang and Melchers, 1947). In developmental terms, vernalization results in acquisition of competence of the meristem to undergo the floral transition. Here we also discussed a model to show how vernalization stably affects competence in the meristem after cold treatment that persists into the spring and throughout the remainder of the life cycle. Molecular mechanism describing gene expression profiling necessary for vernalization is also discussed. Vernalization can be lost as a result of exposure to devernalizing conditions, such as high temperature, but the longer the exposure to low temperature, the more permanent the vernalization effect. Vernalization shortens the vegetative period of the plants, increases the cold resistance of the plants and enhances crop productivity in countries where winter is prolonged.

# Introduction

The promotive effect of low temperature on flowering is termed as **vernalization**. It is also applied to the treatment of seeds and other plant organs at relatively lower temperature.

Vernalization is a process whereby repression of flowering is alleviated by a cold treatment given to a hydrated seed (i.e., a seed that has

imbibed water) or to a growing plant (dry seeds do not respond to the cold treatment because vernalization is an active metabolic process).

Many species of Henbane (*Hyoscyamus sp.*) require vernalization before flowering.

*Arabidopsis thaliana* rosette before vernalization.

## HISTORY

•**John Hancock Klippart**, 1857- first noticed the low temperature requirement for flowering while working with winter wheat and spring wheat.

•**Lysenko**, 1938- published his works on the effects of cold on cereal seeds, and coined the term "Jarovization" (*Jarovoe* in Russian, originally meaning fire or the god of spring) later, translated the term into "Vernalization"

•**P. Chouard**, 1960- defined vernalization as:

**"acquisition or acceleration of the ability to flower by a chilling treatment".**

John Hancock Klippart

P. Chouard

Trofim Lysenko

# CONDITIONS NECESSORY FOR VERNALIZATION:

❖ **Perception of the stimulus:** The cold stimulus is perceived by the apical meristem. However, reports suggest all dividing cells may be the site of vernalization.

❖ **Age of the plant:** Age of plant is important in determination of the responsiveness, varies in species.

❖ **Appropriate low temperature:** Most suitable temperature ranges in between 1º-6ºC. Effective temperature range for vernalization is 0º-10ºC. Temperatures below -6ºC and above 12º-14ºC are ineffective.

❖ **Duration:** Precise duration varies widely with species and variety. Celery as little as 8 days, however 1-3 months of cold treatment is typical.

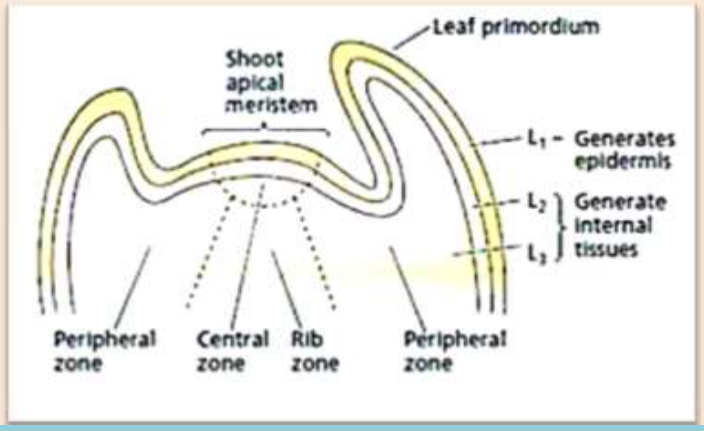
❖ **Necessity of oxygen and water:** Oxygen and water are needed for vernalization.


**Vernalization induces flowering in winter-annual types of *Arabidopsis thaliana*. The plant on the left is a winter-annual type has not been exposed to cold. The plant on the right is a genetically identical winter-annual type that was exposed to 40 days of temperatures slightly above freezing (4ºC) as a seedling. It flowered 3 weeks after the end of the cold treatment with about nine leaves on the primary stem.**

**(Courtesy of Colleen Bizzell.)**

# SITE OF VERNALIZATION

Vernalization appears to take place primarily in the **shoot apical meristem**. Localized cooling causes flowering when only stem apex is chilled and this effect appears to be largely independent of temperature experienced by rest of the plant.




# MECHANISM OF VERNALIZATION

## The two main hypothesis proposed are as follows:

- ❖ Phasic Development Theory
- ❖ Hormonal Hypothesis.

### ❖ PHASIC DEVELOPMENT THEORY:

- **This theory was proposed by Lysenko (1934).**

- This theory proposes growth and development are two distinct phenomenon.
- Process of development of an annual seed plant consists of a series of phases which must occur in some predetermined sequence.

•Commencement of any of these phases will occur only when the preceding phase is completed.

•The phase requires different external conditions for completion.

•Vernalization accelerates the thermophase.

•**For example**: Vernalization of winter wheat accelerates the first cold dependent thermophase.

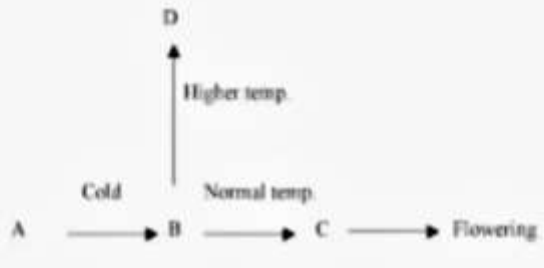
## ❖HORMONAL HYPOTHESIS:

• **This theory was proposed by Melcher (1939).**

• Proposes that perception of the cold stimulus results in the formation of a floral hormone which is transmitted to other plant parts.

• This hormone was named **vernalin**.

**•Lang and Melchers (1947)** proposed a scheme:




According to this scheme, the precursor A is converted into a thermo labile compound B during cold treatment. Under normal conditions B changes into C which ultimately causes flowering. But at higher temperature B is converted into D and flowering does not take place (devernalization)


Vernalization stably affects competence in that there are changes in the pattern of gene expression in the meristem after cold treatment.

## MOLECULAR BASIS OF 'VERNALIZATION'

❖ ***FLC*** (FLOWERING LOCUS C) is a strong repressor of flowering, *FLC* is activated by ***FRI*** (FROST RESISTANCE 1) gene. *FLC* shows high expression in absence of cold treatment, but vernalization represses *FLC*, so flowering may initiate.

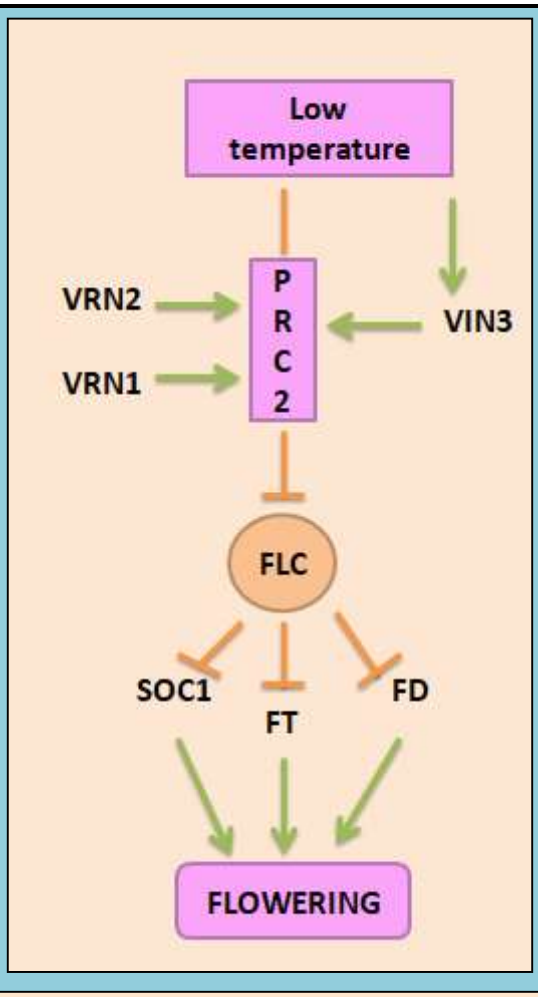
❖ Vernalization is an example of an epigenetic regulation of *FLC* locus.

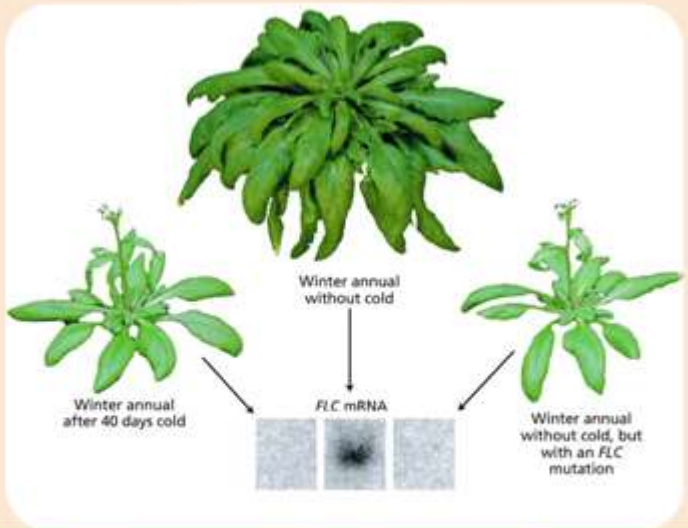
❖ During vernalization treatment, in *Arabidopsis sp.*, ***PRC2*** (POLYCOMB REPRESSIVE COMPLEX) in association with low temperature induced proteins **VIN3** (VERNALIZATION INITIATIVE 3), **VRN1** (VERNALIZATION 1), **VRN2** (VERNALIZATION 2) induces epigenetic regulation of *FLC*.

❖ After vernalization *PRC2* modify histone methylation resulting in repression of *FLC* transcription. FLC being a repressor of flowering ***SOC1*** (SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1) and ***FT*** (FLOWERING LOCUS T) being repressed itself induces flowering.

❖ This process is considered **epigenetic** as the cold treatment induces a **mitotically stable** change, in the gene expression that persists even after cold treatment is withdrawn.

❖ However, the effect is reset during meiosis and progeny do not inherit this state.




**Plants with a vernalization requirement are either quite delayed in flowering or do not flower unless they experience a period of prolonged cold.**

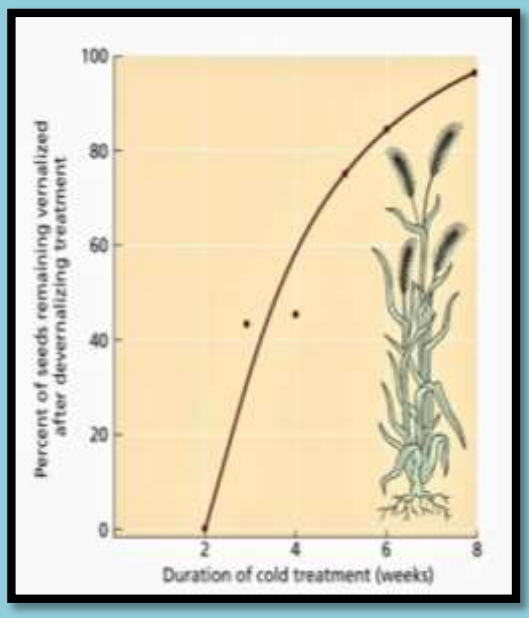
## DEVERNALIZATION

The positive effect of low temperature treatment of the vernalization on plant can be reverseved as a result of exposure to certain conditions

known as **devernalizing conditions** and this phenomenon is known as **devernalization**.

## DEVERNALIZING CONDITIONS ARE:

- In moist seeds or grains by complete drying.
- High temperature (35ºC) treatment.
- Fluctuating low and high temperature.
- Anaerobic conditions.




Duration of exposure to low temperature increases the stability of vernalization effect. The longer that winter rye (*Secale cereale)* is exposed to a cold treatment, the greater the number of plants that remain vernalized when the cold treatment is followed by a devernalizing treatment.

## SIGNIFICANCE OF VERNALIZATION:

- Vernalization shortens the vegetative period of the plants.
- Vernalization increases cold resistance of plant.
- It enhances crop productivity in countries where winter is prolonged.
- It may increase the resistance of plants to disease.

# Conclusion

There does not appear to be a particular vernalization pathway that is conserved among all flowering plants. As discussed previously, FLC is the flowering repressor that is responsible for the vernalization requirement in Arabidopsis. FLC encodes a MADS box protein that is related to regulatory proteins. The epigenetic regulation of FLC involves stable changes in chromatin structure resulting from chromatin remodeling. Vernalization causes the chromatin of FLC gene to lose histone modifications characteristic of euchromatin (transcriptionally active DNA) and to acquire modifications, such as the methylation of specific lysine residues, characteristic heterochromatin (transcriptionally inactive DNA). The cold-induced conversion of FLC from euchromatin to heterochromatin effectively silences the gene.

The activity of FLC underlies many of the physiological features of vernalization-induced flowering in cereals, so understanding how FLC is regulated will provide further insights into the biology of the vernalization response in this plants. A better understanding of the regulation and molecular functions of genes involved in vernalization will also have important implications for cereal breeding programs.

# Acknowledgement

I would like to express my sincere gratitude to Dr. Tripti Roy and Dr. Debjani Sinha Roy, Associate Professors, Botany Department, Bethune

College, Kolkata for giving me such a golden opportunity to present my topic – *'Vernalization: Promoting Flowering with Cold*'. It has been a nice learning experience for me. I would like to cordially thank all my respected departmental teachers for their constant support, guidance and mentorship. Thanks to all my fellow classmates for their support and keeping patience during the presentation.

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# Paper-XI

# Pentose Phosphate Pathway

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# Abstract

The Pentose phosphate pathway (also known as Hexose Monophosphate shunt or Oxidative pentose phosphate pathway) is an alternative pathway to Glycolysis. Its principal product is NADPH, which plays several anabolic roles. The two phases of the pathway shows the conversion of Glucose-6-Phophate to Fructose-6-Phosphate and Glyceraldehyde-3-Phosphate which are in turn used in Glycolysis. This pathway is redox regulated. In this presentation I have tried to explain all the reactions involved in this pathway and the various important enzyme like Transketolase and Transaldolase controlling it are defined along with their function.

# Introduction

Pentose Phosphate Pathway is an alternative pathway to the Glycolytic pathway for glucose / sugar oxidation in plant cells. In plants this pathway is involved in synthesis of glucose in dark reaction of photosynthesis.

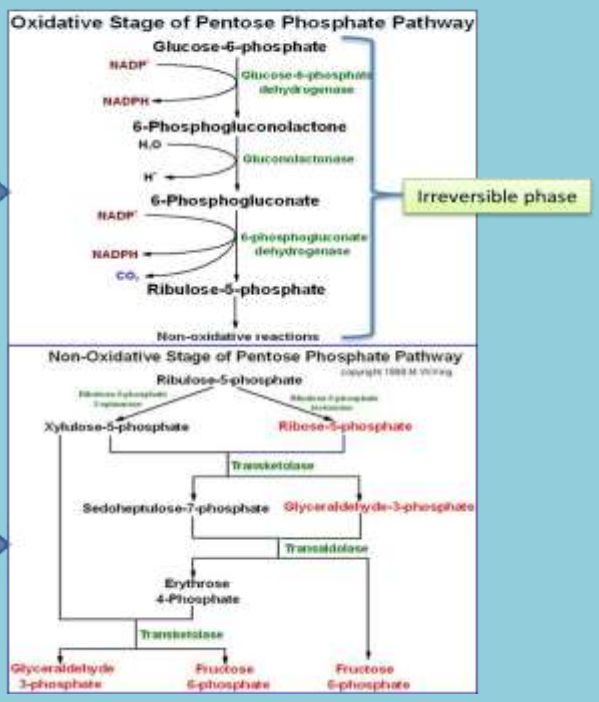
It's principal product is NADPH, which acts as reducing agent in several anabolic processes ,namely, Fatty acid synthesis , cholesterol synthesis, steroid synthesis, assimilation of inorganic nitrogen , etc. While the pentose phosphate pathway does involve oxidation of glucose, its primary role is anabolic rather than catabolic. The pathway is especially important in red blood cells (erythrocytes). The production of NADPH is not linked with the generation of ATP(i.e., no ATP is generated)

The pathway occurs in both cytosol and plastids , although the pathway in plastid predominates over the cytosol pathway. It also occurs in the cytoplasm in case of mammals for example humans.

# THE PATHWAY HAS TWO PHASES :

1. **Oxidative pathway** – 2 molecules of NADPH is generated when glucose-6-phosphate is oxidized to ribulose-5-phosphate . This 5-carbon sugar and its derivates are components of RNA,DNA,ATP,NADH,FAD,CoA. It involves the first three reactions of the pathway. 1 molecule of carbon-dioxide is also produced.
   All the reactions in this phase are irreversible.

2. **Non-Oxidative pathway**- catalyzes the interconversion of 3c, 4c, 5c, 6c and 7c sugars (isomerisation and condensation) in a series of reaction takes place in the cytoplasm. 3 important intermediates of this phase are ribose-5-phosphate, fructose-6-phosphate,glyceraldehyde-3-phosphate.

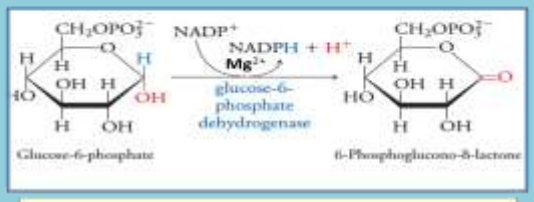



# OXIDATIVE PHASE OF THE PATHWAY

**STEP 1:**

It begins with Oxidation of glucose-6-phosphate to 6-phosphoglucono lactone catalyzed by glucose-6-phosphate dehydrogenase along with coenzyme NADP+ (serves as electron acceptor)which yields one molecule of NADPH by reduction . This is the rate limiting step. Reaction occurs in presence of cofactor $Mg^{2+}$ .

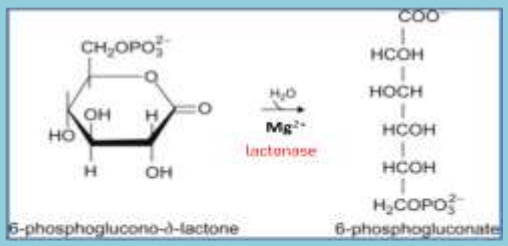
Glucose 6-phosphate + NADP+ ➡ 6-Phosphoglucono-δ-lactone + NADPH + H+




Mechanism: 6-phospho-D-glucono- δ -lactone is an intramolecular ester between C1 carboxyl group and C5 hydroxyl group

**STEP 2:**

The 6-phospho-D-glucono δ -lactone is not stable and the ester suddenly hydrolyses(ring opens) to 6-phospho-D-gluconate.The enzyme which catalyses the reaction is lactonase/ 6-Phosphoglucolactonase Although ring opening occurs in the absence of a catalyst, 6-Phosphogluconolactonase speeds up the reaction, decreasing the lifetime of the highly reactive, and thus potentially toxic, 6-phosphogluconolactone. Reaction occurs in presence of cofactor $Mg^{2+}$




# OXIDATIVE PHASE OF THE PATHWAY


FIG : FIRST TWO REACTIONS OF THE OXIDATIVE PHASE OF PENTOSE PHOSPHATE CYCLE

# OXIDATIVE PHASE OF THE PATHWAY

Step 3 :

In this 6-phospho gluconate further goes through dehydrogenation and oxidative decarboxylation via 6-phosphogluconate dehydrogenase to create the ketopentose, D-ribulose 5-phosphate. The hydroxyl at C3 (C2 of the product) is oxidized to a ketone. This promotes loss of the carboxyl at C1 as $CO_2$. $NADP^+$ again serves as oxidant (electron acceptor) and produces the 2nd molecule of NADPH In this reaction carbon dioxide is also seen to evolve.

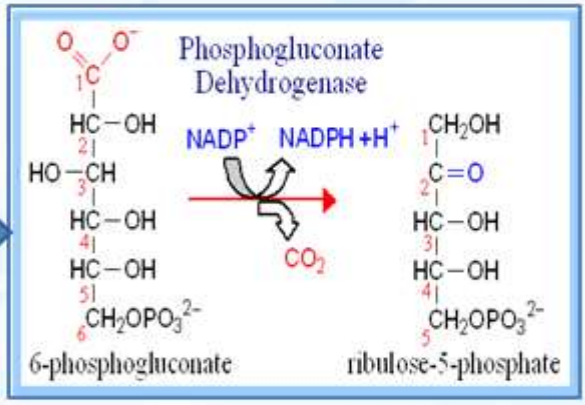




**FIGURE: Reduction of $NADP^+$ involves transfer of $2e^-$ plus $1H^+$ to the nicotinamide moiety.**

# OXIDATIVE PHASE OF THE PATHWAY

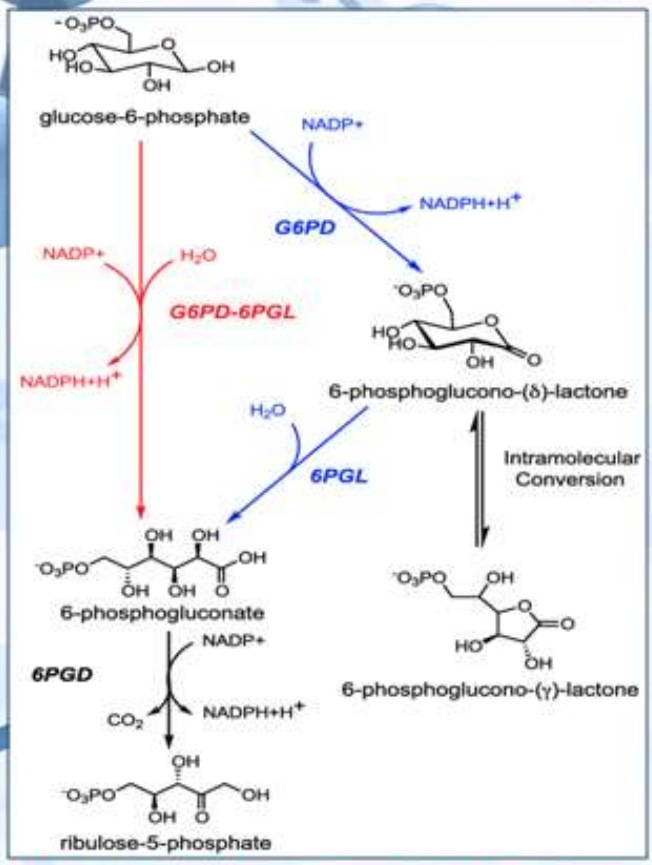



FIG: oxidative phase of the pathway with chemical structures of the products

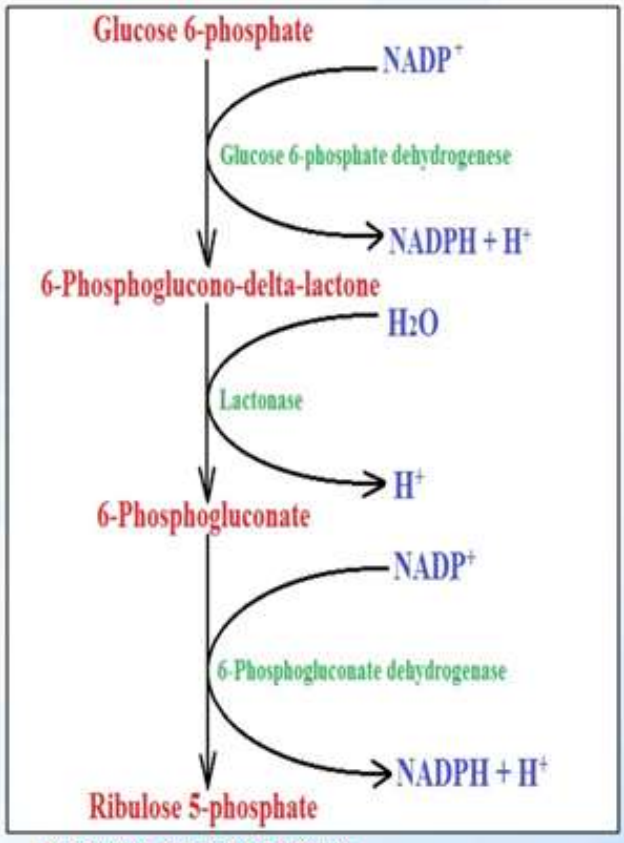
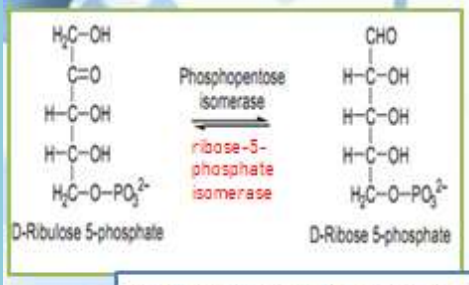



FIG: oxidative phase of the pathway

# NON-OXIDATIVE PHASE OF THE PATHWAY

STEP 1: In the first step ribulose 5-phosphate either isomerizes to ribose-5-phosphate by the enzyme ribose-5-phosphate isomerase or forms xylulose-5-phosphate by epimerization of ribulose 5-phosphate by ribose-5-phosphate epimerase



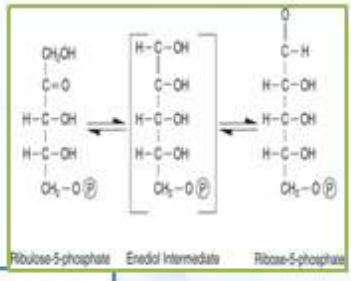




Fig: ribulose 5-phosphate to ribose-5-phosphate

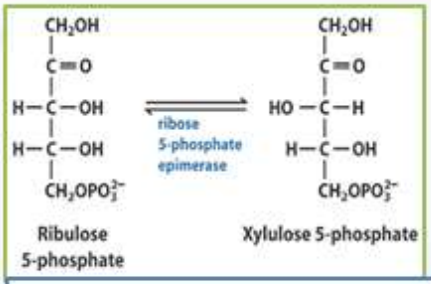
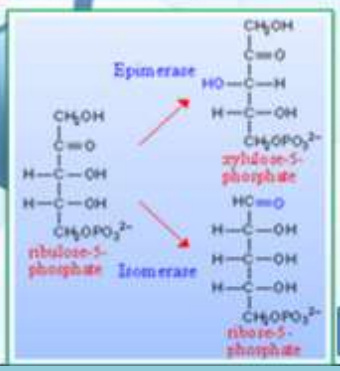



Fig: ribulose 5-phosphate to xylulose-5-phosphate



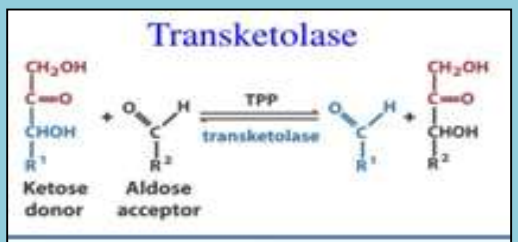

- Epimerase interconverts the stereoisomers ribulose-5-phosphate and xylulose-5-phosphate.
- Isomerase converts the ketose ribulose-5-phosphate to the aldose ribose-5-phosphate.
- Both reactions involve deprotonation to form an endiolate intermediate, followed by specific reprotonation to yield the product. Both reactions are reversible

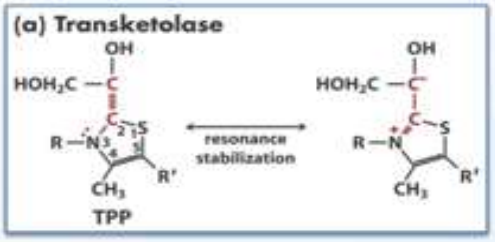
Fig : First step of non-oxidative phase of the pathway

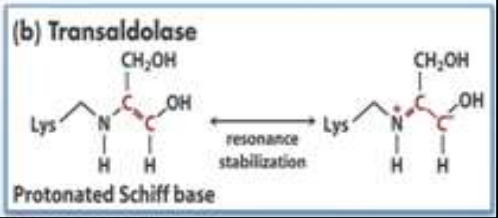
# NON-OXIDATIVE PHASE OF THE PATHWAY

The pentoses are converted into 6- and 3-carbon sugars. The way to decipher it is to remember two key concepts :Either 3-carbon units (one reaction) or 2-carbon units (two reactions) are transferred between acceptor and donor molecules. The enzyme responsible for the 3-carbon transfers is called **Transaldolase**, and the enzyme that is responsible for the transfer of 2-carbon units is called **Transketolase** , in each case from a ketose donor to an aldose acceptor .

The number of carbons involved in the reactions add up to either ten (two reactions) or nine (one reaction). D. E. Nicholson has suggested that the names of these enzymes should be changed, since Transketolase actually transfers an aldol moiety (glycoaldehyde) and Transaldolase actually transfers a ketol moiety (dihydroxyacetone). **(a)Transketolase** is a TPP ( Thiamine pyrophosphate ,a coenzyme form of thiamine also known as vitamin B1) requiring enzyme to do the above mentioned function as TPP stabilizes the 2-carbon carbanion intermediate. In this pathway it catalyzes two reactions **(b) Transaldolase** –This enzyme forms a protonated Schiff base intermediate with a ketose, stabilizing a 3-carbon carbanion intermediate, allowing an aldehyde based sugar to react with the enzyme-linked ketose. The mechanism is similar to aldolase. In this pathway it catalyzes one reaction.



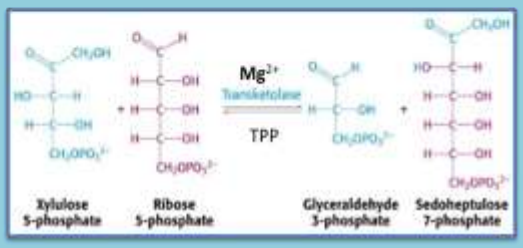



# NON-OXIDATIVE PHASE OF THE PATHWAY

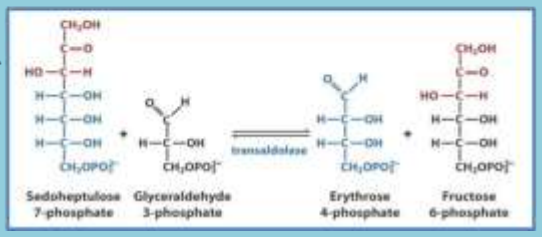
**STEP 2:** In this stage there is reaction of ribose-5-phosphate and xylulose-5-phosphate with transketolase (2-carbon transfer) yields Sedoheptulose 7-phosphate and Glyceraldehyde 3-phosphate (G3P) as shown in the figure below.

The first reaction has the shorthand notation:
5+5=3+7 (2-carbon transfer)




**Step 3 :** the 7-carbon sugar, sedoheptulose-7- phosphate, and the 3-carbon sugar, glyceraldehyde-3-phosphate, react again, in a reaction catalyzed by transaldolase (3-carbon transfer) and yields Fructose-6-phosphate and Erythrose-4-phosphate. Transaldolase cleaves ketose and transfer one of the fragment to a aldose.

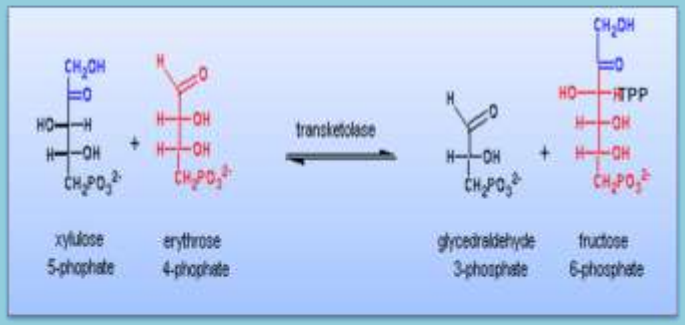
The second reaction has the shorthand notation:
7+3=4+6 (3-carbon transfer)

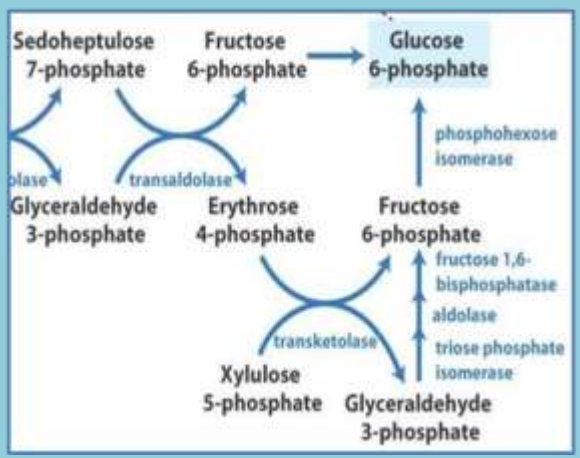



# NON-OXIDATIVE PHASE OF THE PATHWAY

**Step 4 :** The overall conversion in the last reaction, then, is the conversion of two pentoses into a tetrose (4-carbon) molecule and a hexose. Fructose-6-phosphate, the hexose, is a glycolytic intermediate and can enter that pathway at this stage. the 4-carbon sugar, erythrose-4-phosphate, reacts with a molecule of xylulose-5-phosphate, catalyzed by **transketolase** (2-carbon transfer) and yields Fructose-6-phosphate and Glyceraldehyde 3-phosphate (G3P).

The second reaction has the shorthand notation:
4+5=6+3 (2-carbon transfer)



# NON-OXIDATIVE PHASE OF THE PATHWAY

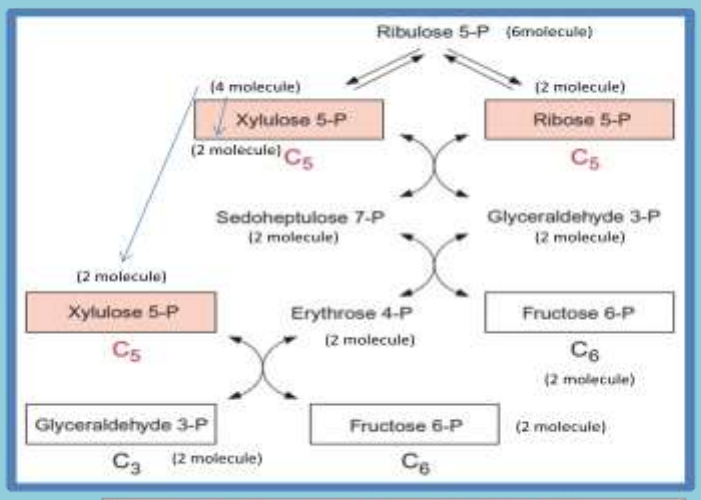



Fig: Non-Oxidative phase of the pathway

## pentose phosphate pathway and glycolysis linked by transketolase and transaldolase

transketolase and transaldolase, this enzymes create a reversible link between pentose phosphate pathway and glycolysis by catalyzing 3 successive reactions of the non oxidative phase:

c5+c5 ⟷ c3+c7( transketolase)
c3+c7 ⟷ c6+c4(transaldolase)
c4+c5 ⟷ c6+c3 (transketolase)
Net result:3 c5 ⟷ 2 c6+ 1 c3.

1) First linked reaction: ribose-5-phosphate + 2 xylulose-5-phosphate ⟷ Sedoheptulose 7-phosphate + Glyceraldehyde 3-phosphate (G3P)

2) Second linked reaction: Sedoheptulose 7-phosphate + Glyceraldehyde 3-phosphate (G3P) ⟷ Fructose-6-phosphate + Erythrose-4-phosphate.

3) Third linked reaction: Erythrose-4-phosphate + xylulose-5-phosphate ⟷ Glyceraldehyde 3-phosphate (G3P) + Fructose-6-phosphate.

The sum of the reactions is :
2 xylulose-5-phosphate+ ribose-5-phosphate ⟷ 2 Fructose-6-phosphate + Glyceraldehyde 3-phosphate (G3P)

# PENTOSE PHOSPHATE PATHWAY

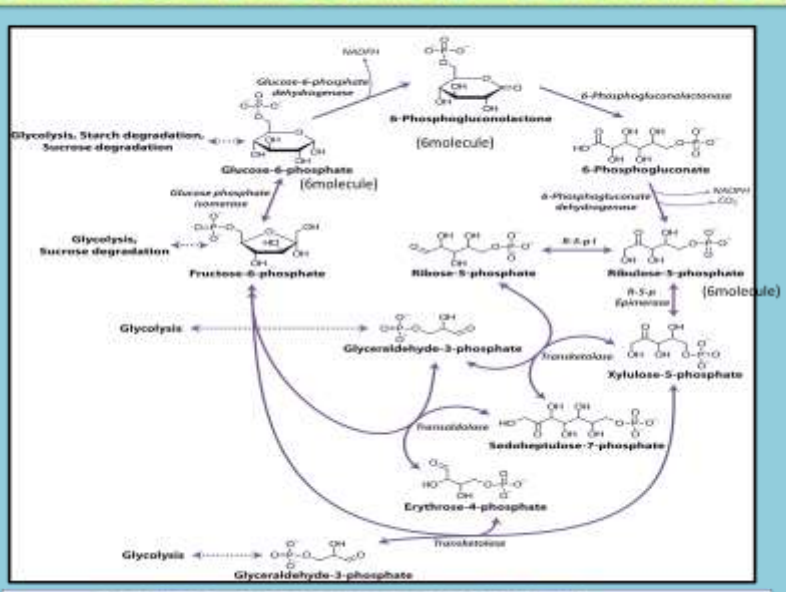



FIG 1: PENTOSE PHOSHATE PATHWAY WITH CHEMICAL STRUCTURES.

# PENTOSE PHOSPHATE PATHWAY

## A. Oxidative phase

1.The pathway begins with the dehydrogenation of glucose-6-phosphate catalyzed by glucose-6-phosphate dehydrogenase to produce 6-phosphoglucolactone and is the first step of the oxidative phase of the pathway.

2.The 6-phosphoglucolactone is then hydrolysed to 6-phosphogluconate by 6-phosphogluconolactonase

3. Then 6-phosphogluconate undergoes oxidative decarboxylation by 6-phosphogluconate dehydrogenase to produce ribulose-5-phosphate in the final step of the oxidative phase.

Overall this phase of the pathway produces two molecules of NADPH from the conversion of glucose-6-phosphate to ribulose-5-phosphate.

## B. Non-Oxidative phase

The non-oxidative phase begins with the reaction of ribulose-5-phosphate with either ribulose-5-phosphate isomerase or ribulose-5-phosphate epimerase followed by a series of reactions catalyzed by transaldolase and transketolase.

These reactions result in the production of two molecules of fructose-6-phosphate and one glyceraldehyde 3-phosphate. The glyceraldehyde 3-phosphate and fructose-6-phosphate in the oxidative pentose phosphate pathway may be exchanged with enzymes of glycolysis.

As with glycolysis, reactions of the pentose phosphate pathway are catalysed by different isoforms of the enzymes that occur either in the cytosol or in plastids. Although transketolase and transaldolase may be absent from the cytosol of some species, the activity is maintained by phosphate translocator proteins on the plastid inner-envelope membrane that have the capacity to translocate **sugar phosphates.**

# STOICHIOMETRY

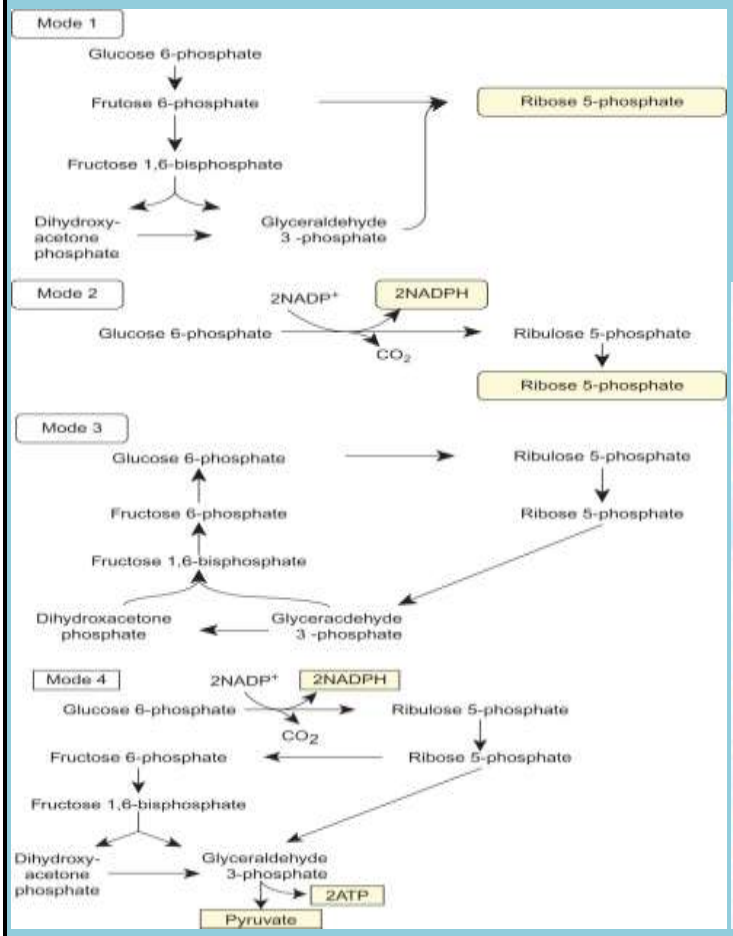



**Figure** -Four modes of the pentose phosphate pathway, each geared to the generation of a product needed by a particular cell.
In mode 1, 1 molecule of glucose-6-phosphate (G6P) makes 5 molecules of ribose-5-phosphate (5R5P).
In mode 2, 1 molecule of G6P makes 1 molecule of R5P and 2 NADPH.
In mode 3, 1 G6P makes 12 NADPH (starting with 6 molecules of G6P oxidized to 6 ribulose 5-phosphates, the ribulose 5-phosphates can be "rearranged by the pathway to form 5 G6Ps." The overall stoichiometry is: **6 G6P + 12 $NADP^+$ → 5 G6P + 12 NADPH + $6CO_2$ + Pi)**.
In mode 4, 3 molecules of G6P make 6 NADPH, 8 ATP and 5 pyruvate and NADH that can be used to create more ATP

## WHY DO WE NEED AN ALTERNATIVE TO GLYCOLYSIS ?

Although glycolysis is the principal route of the conversion of carbohydrates into pyruvic acid in many biological system it is by no means the only known metabolic route for breakdown of carbohydrates .
It has been observed that inhibitors such as Iodoacetate , fluorides, arsenates , etc, which specifically inhibit glucose utilization completely.
This has led to the discovery of some other alternative routes for breakdown of carbohydrates existing in plants, some animal tissues and several types of micro-organisms. One such common alternative is pentose phosphate pathway.

## THE OXIDATIVE PENTOSE PHOSPHATE PATHWAY IS REDOX-REGULATED

The oxidative pentose phosphate pathway is controlled by the initial reaction of the pathway catalyzed by glucose-6-phosphate dehydrogenase, the activity of which is markedly inhibited by a reductive inactivation involving **high ratio of NADPH to NADP+** and **ferredoxin–thioredoxin** system thus inhibited during photosynthesis due to high ratio in chloroplast.

In the light, however, little operation of the oxidative pathway is likely to occur in the chloroplast because the end products of the pathway, fructose-6-phosphate and glyceraldehyde-3-phosphate, are being synthesized by the Calvin cycle. Thus, mass action will drive the nonoxidative interconversions of the pathway in the direction of pentose synthesis, i.e., in the reverse direction. In this way, synthesis of erythrose-4-phosphate can be maintained in the light. In non-green plastids, the glucose-6-phosphate dehydrogenase is less sensitive to inactivation by reduced thioredoxin and NADPH , and can therefore reduce NADP+ to maintain a high reduction of plastid components in absence of photosynthesis .

## SIGNIFICANCE OF OXIDATIVE PENTOSE PHOSPHATE PATHWAY

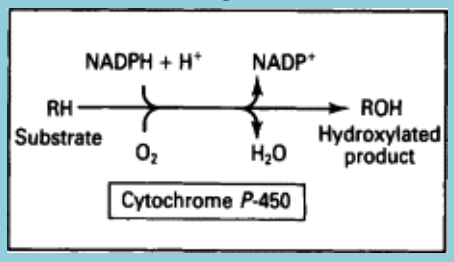
The oxidative pentose phosphate pathway plays several roles in plant metabolism:

1. The product of the two oxidative steps is NADPH, and this NADPH is thought to drive reductive steps associated with various biosynthetic reactions that occur in the cytosol. In nongreen plastids, such as amyloplasts, and in chloroplasts functioning in the dark, the pathway may also supply NADPH for biosynthetic reactions such as lipid biosynthesis and nitrogen assimilation. NADPH is passed to cytoplasm and plastid in absence of photosynthesis.

2. Because plant mitochondria are able to oxidize cytosolic NADPH via an NADPH dehydrogenase localized on the external surface of the inner membrane, some of the reducing power generated by this pathway may contribute to cellular energy metabolism; that is, electrons from NADPH may end up reducing O2 and generating ATP.

3. The pathway produces ribose-5-phosphate, a precursor of the ribose and deoxyribose needed in the synthesis of RNA and DNA, respectively.

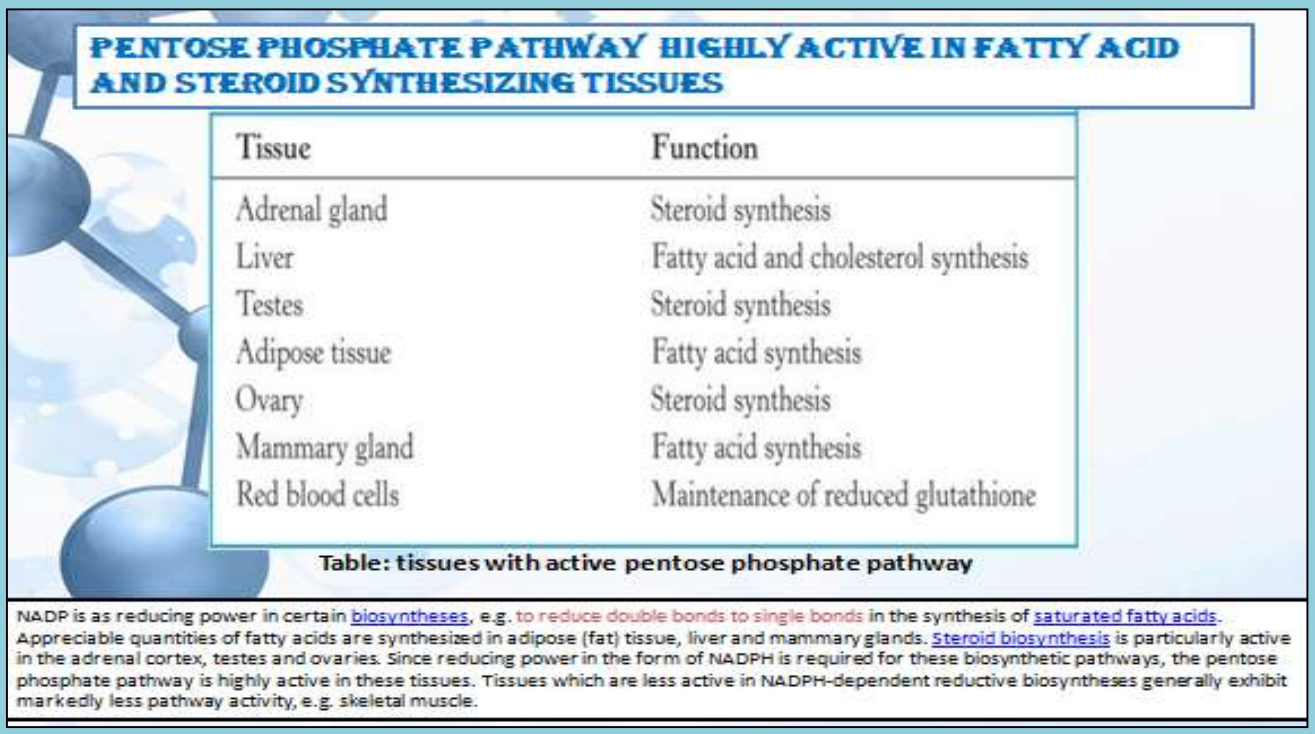
4. Another intermediate in this pathway, the four-carbon erythrose-4-phosphate, combines with PEP in the initial reaction that produces plant phenolic compounds, including the aromatic amino acids and the precursors of lignin, flavonoids, and phytoalexins.
5. During the early stages of greening, before leaf tissues become fully photoautotrophic, the oxidative pentose phosphate pathway is thought to be involved in generating Calvin cycle intermediates.
6. The reversible oxidative section of the pathway is the source of carbon skeletons for the synthesis of a number of compounds. For example ribose-5-phosphate provides the ribosyl moiety of nucleotides and is a precursor for the biosynthesis of purine skeletons and erythrose-4-phosphate, which is the precursor for the biosynthesis of aromatic amino acids by the shikimic acid pathway.( as mentioned in point 4)




7. pentose phosphate pathway is responsible for detoxification of blood by reducing the oxidized glutathione and detoxification of cytochrome P450 mono oxygenases . Moreover , reactive oxygen species (ROS) generated in oxidative metabolism inflict the damage on all classes of macromolecule leading to many diseases and even death of the organism. This oxidative stress is reduced by NADPH (being a strong antioxidant, maintains redox potential) generated by pentose phosphate pathway , for example, glutathione reduction. Reduced glutathione helps prevent the oxidation of the iron in haemoglobin from Fe(II) to Fe(III). Haemoglobin containing Fe(III) is not effective in binding $O_2$.Also protects against cell lysis due to the oxidation of unsaturated lipids of the cell membrane.
8. The metabolism of glucose-6-phosphate by the pentose phosphate pathway is coordinated with glycolysis. The glycolytic pathway and

pentose phosphate pathway enables the level of NADPH, ATP, and building blocks such ribose-5-phosphate and pyruvate to be continuously adjusted to meet cellular needs , for example excess ribose-5-phosphate formed by pentose phosphate pathway can be converted into glycolytic intermediates like fructose-6-phosphateand glyceraldehyde-3-phosphate or vice versa

9. carbon-dioxide molecule from isotopically labeled glucose shows that pentose phosphate pathway carries out 10-25% of glucose breakdown, rest via glycolysis.



**PENTOSE PHOSPHATE PATHWAY HIGHLY ACTIVE IN FATTY ACID AND STEROID SYNTHESIZING TISSUES**

| Tissue | Function |
|---|---|
| Adrenal gland | Steroid synthesis |
| Liver | Fatty acid and cholesterol synthesis |
| Testes | Steroid synthesis |
| Adipose tissue | Fatty acid synthesis |
| Ovary | Steroid synthesis |
| Mammary gland | Fatty acid synthesis |
| Red blood cells | Maintenance of reduced glutathione |

**Table: tissues with active pentose phosphate pathway**

NADP is as reducing power in certain biosyntheses, e.g. to reduce double bonds to single bonds in the synthesis of saturated fatty acids. Appreciable quantities of fatty acids are synthesized in adipose (fat) tissue, liver and mammary glands. Steroid biosynthesis is particularly active in the adrenal cortex, testes and ovaries. Since reducing power in the form of NADPH is required for these biosynthetic pathways, the pentose phosphate pathway is highly active in these tissues. Tissues which are less active in NADPH-dependent reductive biosyntheses generally exhibit markedly less pathway activity, e.g. skeletal muscle.

# Conclusion

The pentose phosphate pathway in animals fulfills two important cell requirements: 1) for ribose 5-phosphate for the synthesis of nucleotides and nucleic acids; and 2) for reducing power in the form of NADPH. In photosynthesis, it functions to regenerate the primary CO2 acceptor, ribulose bisphosphate, from the hexose phosphates produced. Chloroplasts utilize radiant energy to produce ATP, required for the production of ribulose 1,5-bisphosphate from ribulose 5-phosphate and

also for the reduction of 3-phosphoglyceric acid to glyceraldehyde 3-phosphate. The reducing agent for the latter reaction, NADPH, is also generated by the action of light in the chloroplasts. In both animals and plants, NADP rather than NAD appears to function as the coenzyme for reductive synthesis.

# Acknowledgement

I would like to express my gratitude towards Dr. Tripti Roy and Dr. Debjani Sinha Roy, associate professors of Botany department, Bethune college for giving me this golden opportunity of making a presentation on this topic. This enlightened me on a number of new things.

I am also thankful to my fellow classmates for their support and encouragement.

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# Paper-XII

# Mineral Nutrient Deficiency Symptoms In Plants


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# Abstract

Mineral Deficiency Symptoms in a plant are the expression of metabolic disorders resulting from the insufficient supply of an essential mineral element. Essential mineral elements are usually classified as macronutrients , micronutrients, mobile nutrients and immobile nutrients.The nutrients that can cause deficiency symptoms in plants are nitrogen, sulphur, phosphorus, silicon, boron, potassium, sodium, calcium, chlorine, magnesium zinc, iron , manganese copper, nickle and molybdenum. Nutritional deficiencies can be treated by addition of fertilizers in the crop.

# Introduction

Nutrient deficiency symptoms in a plant are the expression of metabolic disorders resulting from the insufficient supply of an **essential mineral element**. These disorders are related to the roles played by essential elements in normal plant metabolism and function.

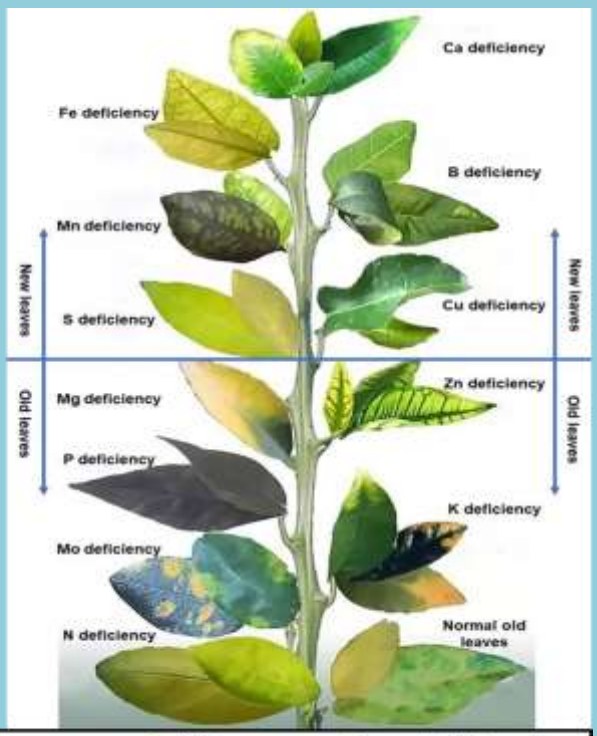



Fig.: Visual appearance of different nutrient deficiencies

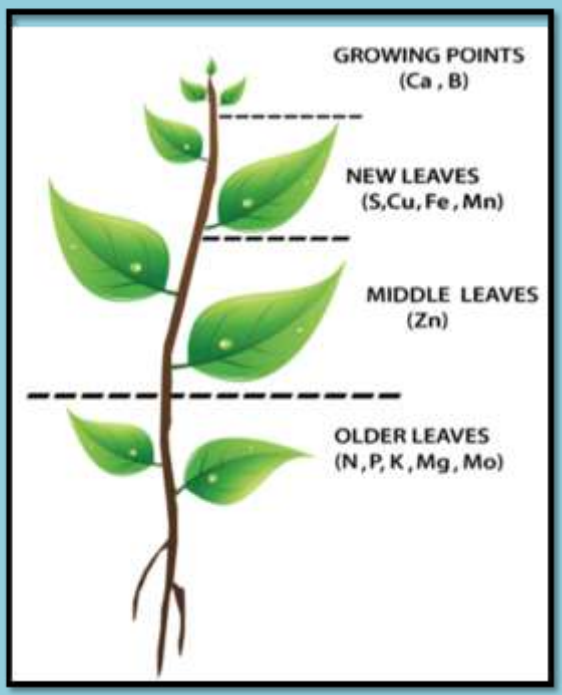
# Classification of Nutrient Deficiency Symptoms

**Nutrient deficiency symptoms may be classified as follows:**

- ❖ Complete crop failure at the seedling stage.
- ❖ Severe stunting of plants.
- ❖ Specific leaf symptoms appearing at varying times during the season.
- ❖ Internal abnormalities such as clogged conductive tissues.
- ❖ Delayed or abnormal maturity.
- ❖ Obvious yield differences, with or without leaf symptoms.
- ❖ Poor quality of crops, including differences in protein, oil, or starch content, and storage quality.
- ❖ Yield differences detected only by careful experimental work.

## What are Essential Mineral Elements?

Plants obtain carbon, hydrogen and oxygen from water, air and sunlight, to make food for growth.
Essential mineral elements are usually classified as *macronutrients* or *micronutrients* according to their relative concentrations in plant tissue.




### Macronutrients

**Namely:** Nitrogen, phosphorus, potassium, sulphur, calcium and magnesium. These are required by plants in relatively large amounts.

### Micronutrients

**Namely:** Copper, manganese, zinc, iron, boron and molybdenum. These are required by plants in smaller quantities.

Mineral elements are also classified on the basis of their mobility within a plant and their tendency to retranslocate during deficiencies.

## Mobile Nutrients

If an essential element is mobile, deficiency symptoms tend to appear first in older leaves.
**Namely:** Nitrogen, Potassium, Magnesium, Phosphorous, Chlorine, Sodium, Zinc, Molybdenum

## Immobile Nutrients

If an essential element is immobile, deficiency symptoms tend to appear first in younger leaves.
**Namely:** Calcium, Sulphur, Iron, Boron, Copper

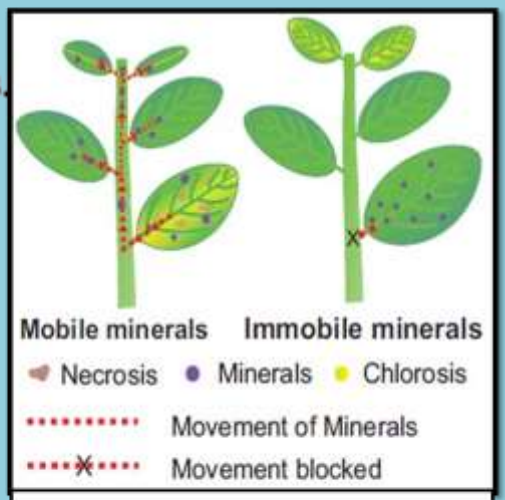


Fig.: Mobility of Minerals

# Functions of Essential Nutrients and their Deficiency Symptoms:

| (GROUP 1) | NITROGEN(N) |
|---|---|
| **Function:** | Nitrogen is needed by plants to promote rapid **growth** especially for fruit and seed **development.** Also, it increases leaf size and quality, and hastens plant maturity. |
| **Deficiency :** | General **chlorosis** of entire plant to a light green followed by yellowing of older leaves proceeding towards younger leaves. Plants become **spindly, stunted and secondary shoots develop poorly** if the initial symptoms are not corrected. |
| |  **Whole leaves turn yellow, starting from the lower to upper leaves.** |

| (GROUP2) | SULPHUR(S) |
|---|---|
| **Function:** | Sulphur is found in certain amino acids and is a constituent of several coenzymes and vitamins, such as coenzyme A, S-adenosylmethionine, biotin, vitamin B1, and pantothenic acid, which are **essential for metabolism** |
| **Deficiency:** | Many of the symptoms of sulphur deficiency are **similar to those of nitrogen deficiency**, including **leaf chlorosis, stunting of growth**, and anthocyanin accumulation. |

| (GROUP 2) | PHOSPHOROUS(P) |
|---|---|
| **Function:** | Phosphorus is needed by plants to promote **photosynthesis, protein formation, seed germination, bloom stimulation and budding**. It also hastens **maturity.** |
| **Deficiency:** | Characteristic symptoms include **stunted growth** of the entire plant and a **dark green coloration of the leaves**, which may be malformed and contain small areas of dead tissue called **necrotic spots.** |
| |  **Necrotic spots on the leaf surface due to Phosphorous deficiency.** |

| (GROUP 2) | SILICON(Si) |
|---|---|
| **Function:** | Deposited as amorphous silica in cell walls. Contributes to cell wall mechanical properties, including **rigidity** and **elasticity.** |
| **Deficiency:** | Plants deficient in silicon are more susceptible to **lodging** (falling over) and **fungal infection.** |

| (GROUP 2) | BORON(B) |
|---|---|
| **Function:** | Boron is needed in the process of **cell differentiation** at the growing tips of plants where cell division is active. |
| **Deficiency:** | **Black necrosis** of young leaves and terminal buds. Stems may be unusually **stiff** and **brittle**. **Apical dominance may also be lost**, causing the plant to become **highly branched**; however, the terminal apices of the branches and Structures such as the fruits, fleshy roots, and tubers may exhibit **necrosis** or **abnormalities.** |
| |  **Leaf buds are discoloured. They will break and drop eventually.** |

| (GROUP 3) | POTASSIUM(K) |
|---|---|
| **Function:** | It plays an important role in regulation of the **osmotic potential** of plant cells. It also activates many **enzymes involved in respiration and photosynthesis**. |
| **Deficiency:** | **Mottled or marginal chlorosis**, which then develops into necrosis primarily at the leaf tips, at the margins, and between veins. The leaves may also **curl** and **crinkle.** The stems of potassium-deficient plants may be slender and weak, with abnormally short internodal regions. |
| |  **Browning or Yellowing on Leaf Edges of newly matured leaves.** |

| (GROUP 3) | SODIUM(Na) |
|---|---|
| **Function:** | Involved with the **regeneration of phosphoenolpyruvate** in C4 and CAM plants. **Substitutes for potassium** in some functions |
| **Deficiency:** | Under sodium deficiency, these plants exhibit **chlorosis** and **necrosis**, or even **fail to form flowers.** |

| (GROUP 3) | CALCIUM(Ca) |
|---|---|
| **Function:** | Calcium is needed by plants to produce new growing points and root tips. Calcium ions have two distinct roles in plants: (1) a **structural/apoplastic** role and (2) a **signalling role.** |
| **Deficiency:** | **Necrosis** of young meristematic regions such as the tips of roots or young leaves, etc. Necrosis may be preceded by a **general chlorosis** and **downward hooking** and **deformation of young leaves**. The root system may appear brownish, short, and highly branched. |
| |  **New leaves are paler with stunted growth as compared to the older leaves.** |

| (GROUP 3) | CHLORINE(Cl) |
|---|---|
| **Function:** | It is required for the **water-splitting reaction of photosynthesis** through which oxygen is produced and for cell division in leaves and roots. |
| **Deficiency:** | **Wilting** of the leaf tips followed by general **leaf chlorosis** and **necrosis**. Eventually, the leaves may take on a bronze like colour ("**bronzing**").  **Purple or Bronze discoloration on the older leaves** |

| (GROUP 3) | MAGNESIUM(Mg) |
|---|---|
| Function: | It has a specific role in the activation of enzymes involved in **respiration, photosynthesis**, and the **synthesis of DNA and RNA**. It is also part of the ring structure of the chlorophyll molecule. |
| Deficiency : | **Chlorosis between the leaf veins**, occurring first in older leaves. If the deficiency is extensive, the leaves may become yellow or white. **Senescence** and **premature leaf abscission** may also occur. |
| |  **Lower leaves are paler and chlorotic as compared to upper leaves, with dark green veins. This is known as interveinal chlorosis.** |

| (GROUP 3) | ZINC(Zn) |
|---|---|
| Function: | Many enzymes require zinc ions for their activity, and zinc may be required for **chlorophyll biosynthesis** in some plants. |
| Deficiency : | **Reduction in internodal growth**, and as a result plants display a **rosette habit of growth**. The leaves may also be small and distorted, with leaf margins having a **puckered appearance**.  **Yellowing between veins.** |

| (GROUP 4) | IRON(Fe) |
|---|---|
| Function: | Required for the **synthesis of chloroplast proteins** and various **enzymes** involved in the transfer of electrons, such as cytochromes. |
| Deficiency : | Light green to yellow **interveinal chlorosis** on newly emerging leaves and young shoots. It is common to see shoots dying from the tip inwards. In severe cases, newly emerged leaves may reduce in size and turn nearly **white**, with **necrotic spots**. |
| |  **Young leaves are paler as compared to matured leaves, with dark green veins.** |

| (GROUP 4) | MANGANESE(Mn) |
|---|---|
| Function: | It is needed by plants for **photosynthesis**, **respiration** and activation of several enzymes in plant cells. |
| Deficiency : | **Intervenous chlorosis** associated with small **necrotic spots**. In severe deficiency, new leaves become smaller and **tip dieback** can occur.  **Yellowing between veins and broad green areas around veins around younger leaves** |

| (GROUP 4) | COPPER(Cu) |
|---|---|
| **Function:** | Like iron, copper is associated with enzymes involved in redox reactions, through which it is reversibly oxidized. |
| **Deficiency:** | Production of **dark green leaves**, which may contain **necrotic spots**. The necrotic spots appear first at the tips of young leaves and then extend toward the leaf base along the margins. The leaves may also be **twisted** or **malformed**. Under extreme copper deficiency, **leaves may drop prematurely** and **flowers may be sterile.** |
| **(GROUP 4)** | **NICKEL(Ni)** |
| **Function:** | Constituent of urease. In N2-fixing bacteria, constituent of hydrogenases. |
| **Deficiency:** | Nickel-deficient plants accumulate urea in their leaves and consequently show **leaf tip necrosis.** |
| **(GROUP 4)** | **MOLYBDENUM(Mo)** |
| **Function:** | Constituent of nitrogenase, nitrate reductase, and xanthine dehydrogenase. |
| **Deficiency:** | General **chlorosis** between veins and **necrosis** of older leaves. The leaves may not become necrotic but instead may appear twisted and subsequently die (**whiptail disease**). Flower formation may be prevented, or the **flowers may abscise prematurely**. |

# Conclusion

Nutrient deficiency symptoms appear only after the nutrient supply is so low that the plants can no longer function properly. If the symptoms are observed early, it might be corrected during the growing season. Usually the yield is reduced below the quantity that would have been obtained if adequate nutrients had been available at the beginning. However, if the problem is properly diagnosed, the deficiency can be corrected.

**Crop yields can be improved by the addition of fertilizers:**

❖ Most chemical fertilizers contain inorganic salts of the macronutrients nitrogen, phosphorus, and potassium.

❖ Adding micro-nutrients to the soil may also be necessary to correct a pre-existing deficiency.

❖ Organic fertilizers are also used for nutritional deficiencies.

**Some mineral nutrients can be absorbed by leaves:**

❖ In addition to absorbing nutrients added to the soil as fertilizers, most plants can absorb mineral nutrients applied to their leaves as a spray, a process known as foliar application.

❖ Nutrient uptake by leaves is most effective when the nutrient solution is applied to the leaf as a thin film.

❖ Addition of lime to the spray diminishes the solubility of many nutrients and limits toxicity.

❖ In wheat (*Triticum aestivum*), nitrogen applied to the leaves during the later stages of growth enhances the protein content of seeds.


# Acknowledgement

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convey my thanks to all my fellow classmates for guiding me, without which this presentation would have been incomplete.

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# Paper-XIII

# The Citric Acid Cycle

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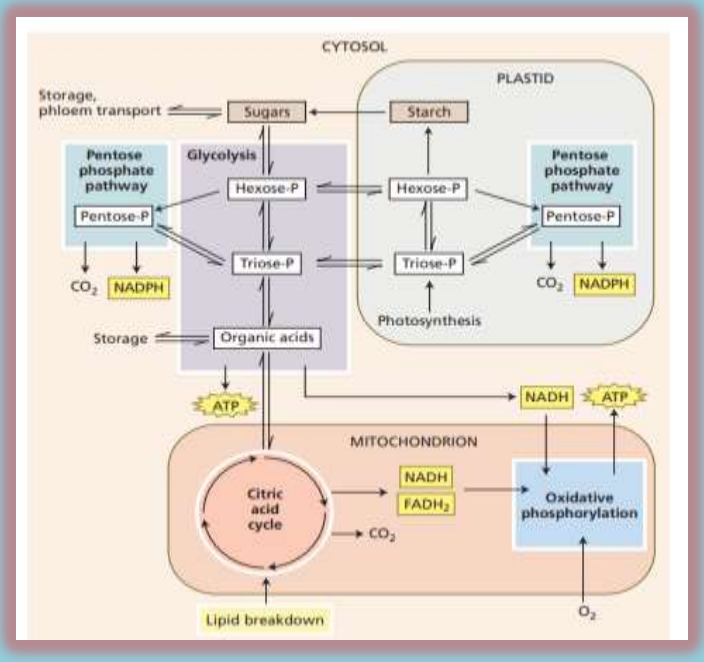
# Abstract

The citric acid cycle (Krebs cycle or tricarboxylic acid—TCA cycle) is the most important metabolic pathway for the energy supply to the body. About 65-70% of the ATP is synthesized in Krebs cycle. Citric acid cycle essentially involves the oxidation of acetyl CoA to CO2 and H2O. This cycle utilizes about twothirds of total oxygen consumed by the body. The name TCA cycle is used, since, at the outset of the cycle, tricarboxylic acids (citrate, cisaconitate and isocitrate) participate. Krebs cycle basically involves the combination of a two carbon acetyl CoA with a four carbon oxaloacetate to produce a six carbon tricarboxylic acid, citrate. In the reactions that follow, the two carbons are oxidized to CO2 and oxaloacetate is regenerated and recycled. Oxaloacetate is considered to play a catalytic role in citric acid cycle. In this presentation I have tried to explain all the reactions involved in this cycle and the enzymes controlling it.

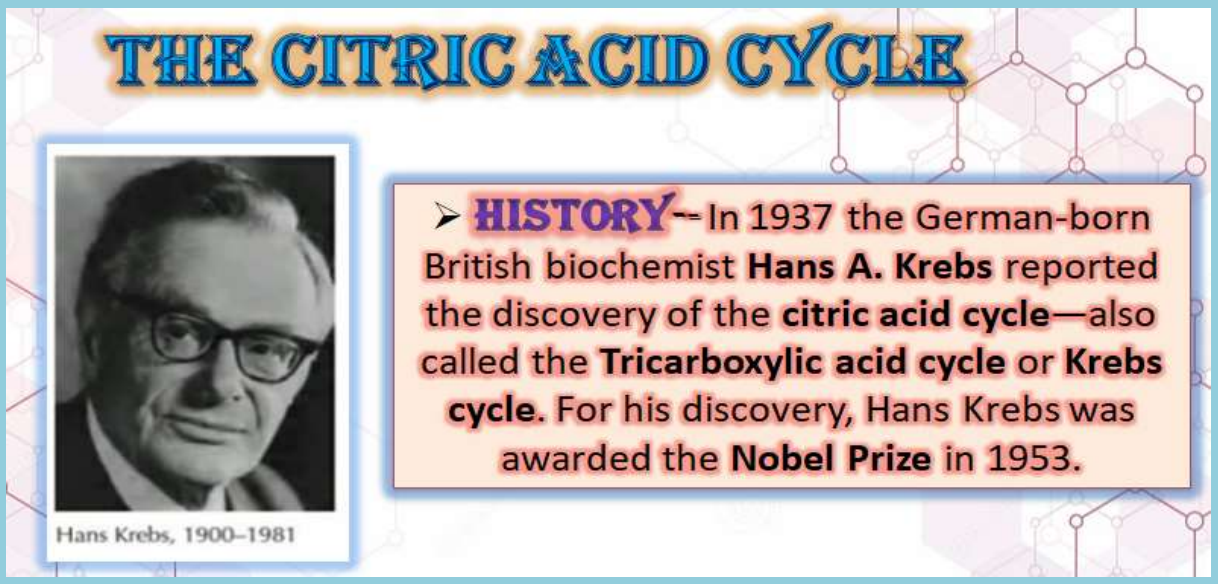
# Introduction

The cell oxidizes glucose or sucrose in a series of step-by-step reactions.



These reactions can be grouped into four major processes:

1. Glycolysis
2. **The Citric Acid Cycle**
3. Oxidative Phosphorylation
4. The Oxidative Pentose Phosphate Pathway




**Definition** -- The citric acid cycle is a series of chemical reactions that oxidize **organic acids** to produce **carbondioxide($CO_2$)** and transfers the resulting electrons to reduce the redox cofactors **$NAD^+$** (Nicotinamide Adenine Dinucleotide) and **FAD**(Flavin Adenine Dinucleotide), forming **NADH** and **$FADH_2$**

**Site of occurrence** -- Mitochondrial Matrix

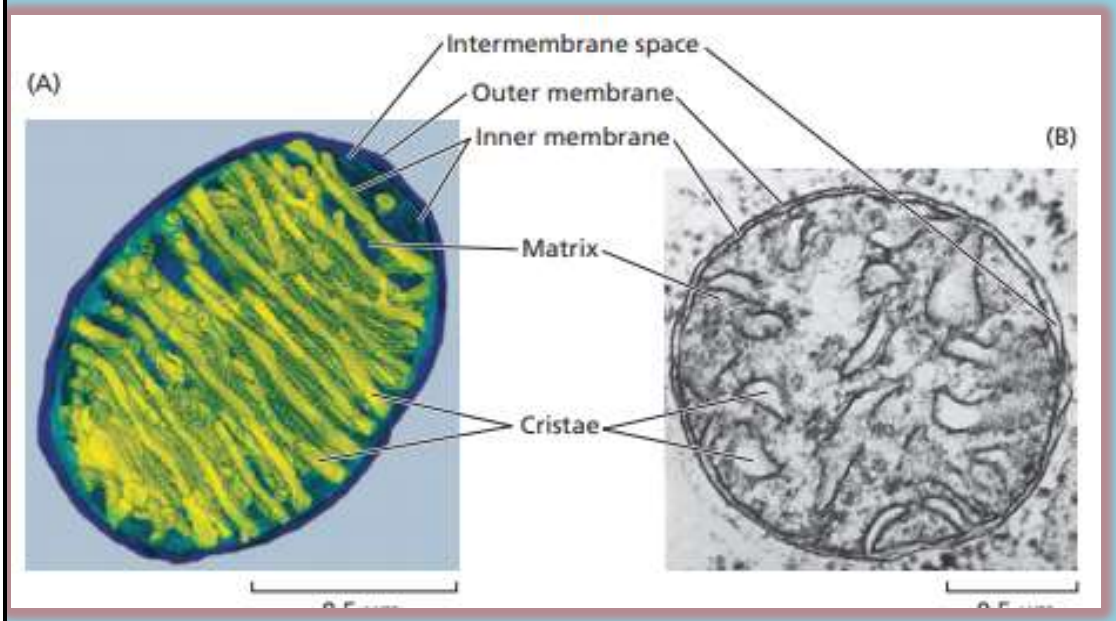
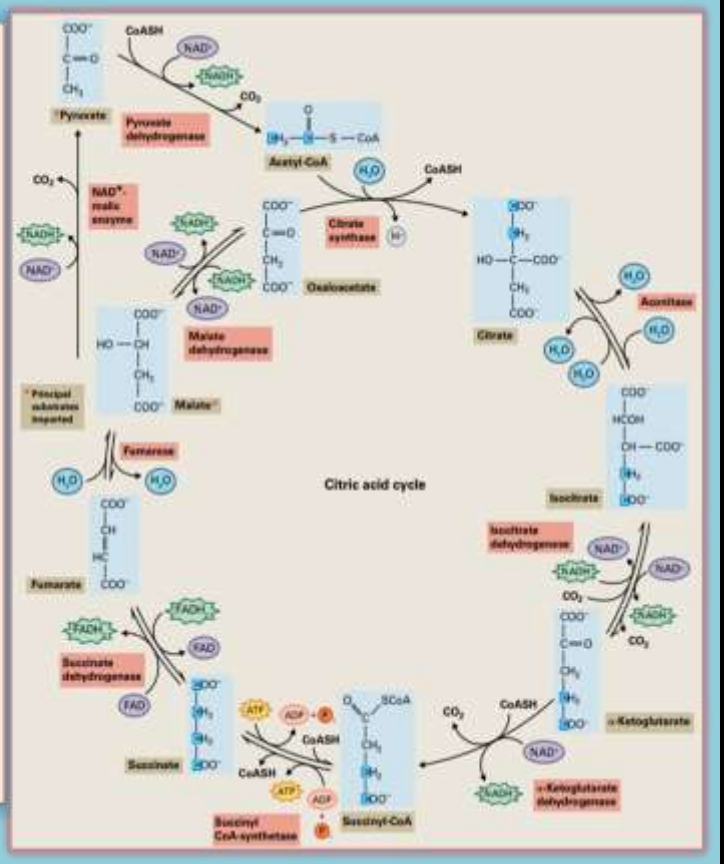



Fig.: Structure of Mitochondria

# REACTIONS OF THE CITRIC ACID CYCLE

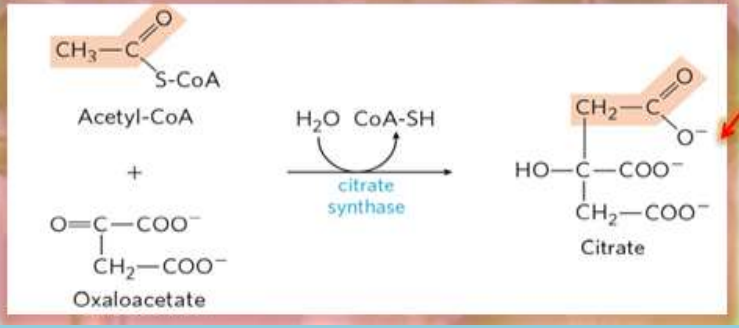
- The citric acid cycle is also known as the **tricarboxylic acid cycle** because of the importance of the tricarboxylic acids- **citric acid** and **isocitric acid** as early intermediates .
- The **pyruvate** generated in the cytosol during glycolysis be transported through the impermeable inner mitochondrial membrane.
- Once inside the mitochondrial matrix, pyruvate is **decarboxylated** in an oxidation reaction catalyzed by **pyruvate dehydrogenase.**
- The products are **NADH, $CO_2$,** and **acetyl-CoA,** in which the acetyl group derived from pyruvate is linked by a **thioester bond** to a cofactor, **coenzyme A (CoA)**

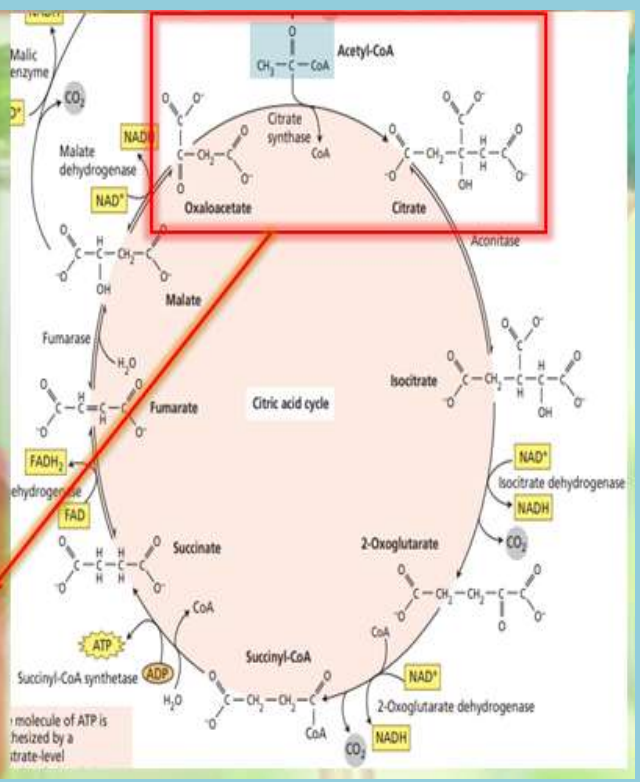



# THE CITRIC ACID CYCLE HAS EIGHT STEPS

## STEP 1- FORMATION OF CITRATE

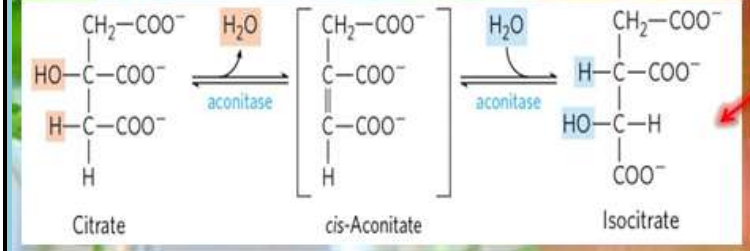
- **Substrate:-** Oxaloacetate,Acetyl-CoA
- **Product:-** Citrate, CoA-SH
- **Enzyme:** Citrate synthase
- **Type of Reaction:** Condensation (Irreversible Reaction)

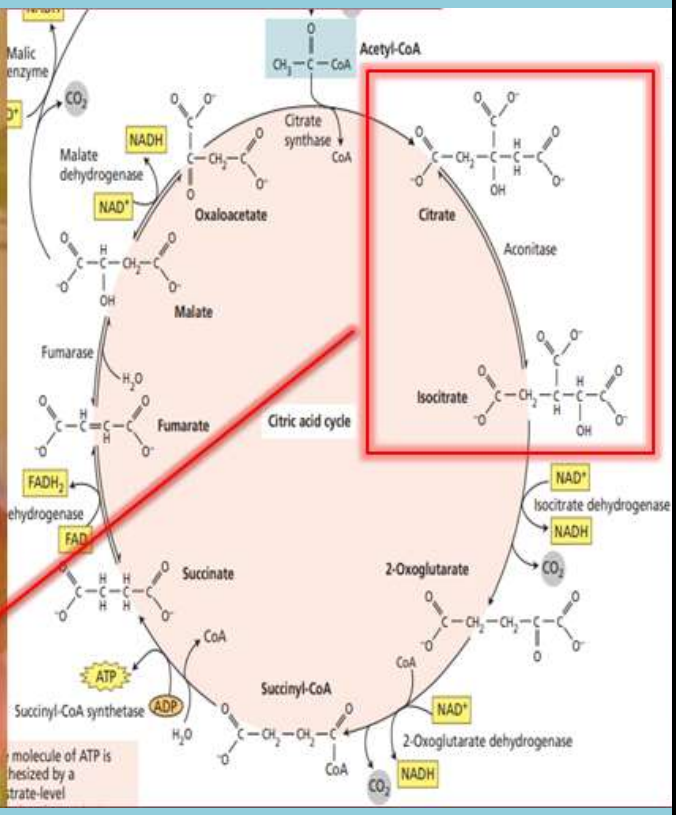


## STEP 2- FORMATION OF ISOCITRATE

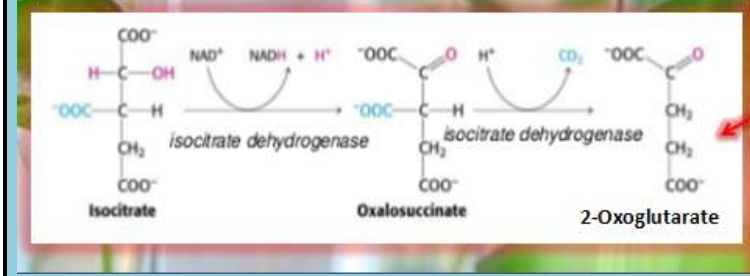
- **Substrate:-** Citrate
- **Product:-** Isocitrate
- **Enzyme:** Aconitase
- **Type of Reaction:** Dehydration (Reversible Reaction)

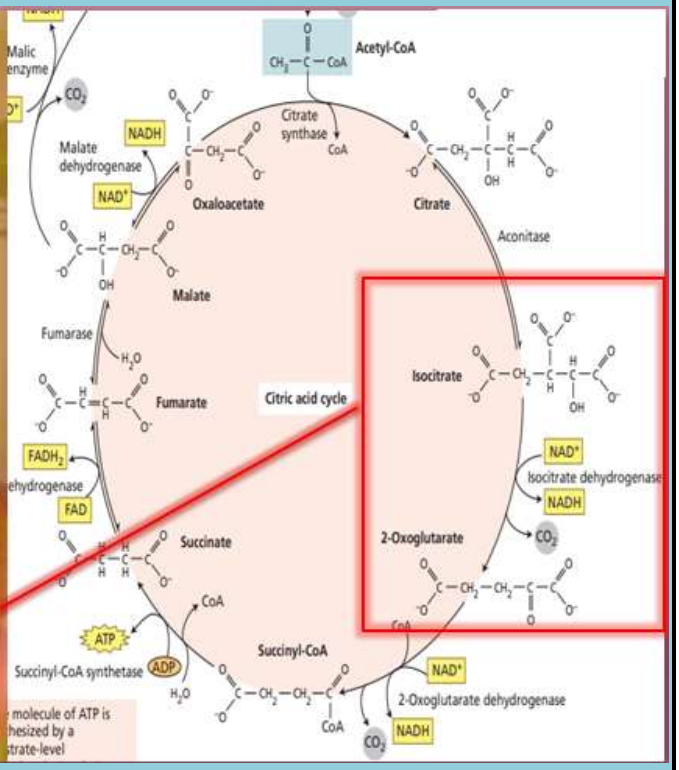



## STEP 3- OXIDATION OF ISOCITRATE TO 2-OXOGLUTARATE AND $CO_2$

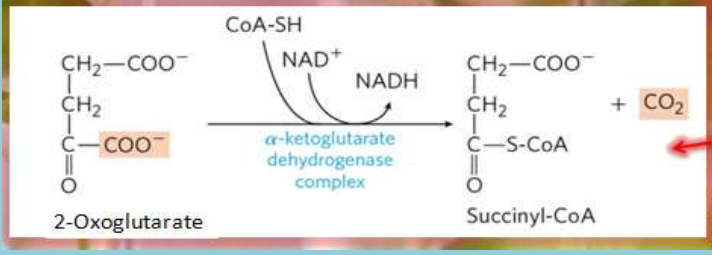
- **Substrate:-** Isocitrate
- **Product:-** 2-Oxoglutarate, NADH, $CO_2$
- **Enzyme:** Isocitrate dehydrogenase
- **Cofactor:** $NAD^+$
- **Type of Reaction:** **O**xidative decarboxylation(Irreversible Reaction)

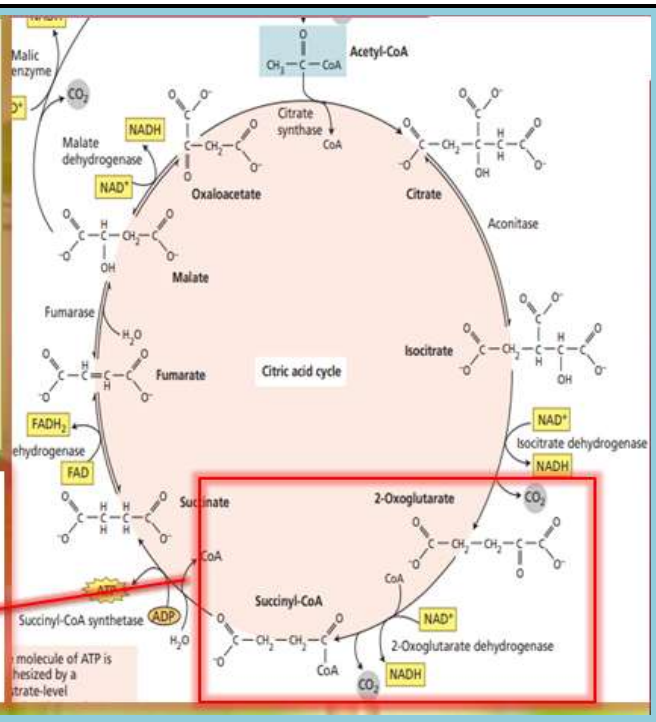


## STEP 4- OXIDATION OF 2-OXOGLUTARATE TO SUCCINYL-COA AND $CO_2$

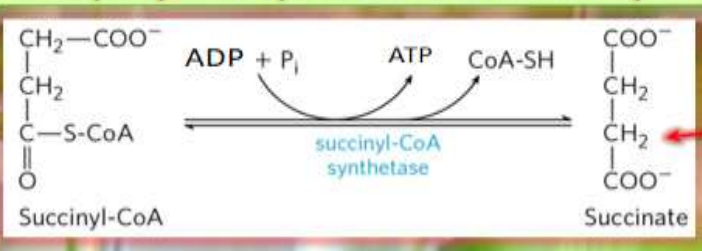
- **Substrate:-** 2-Oxoglutarate
- **Product:-** Succinyl-CoA, $CO_2$, NADH
- **Enzyme:** 2-Oxoglutarate dehydrogenase
- **Cofactor:** $NAD^+$
- **Carrier:** CoA
- **Type of Reaction:** **O**xidative decarboxylation(Irreversible Reaction)

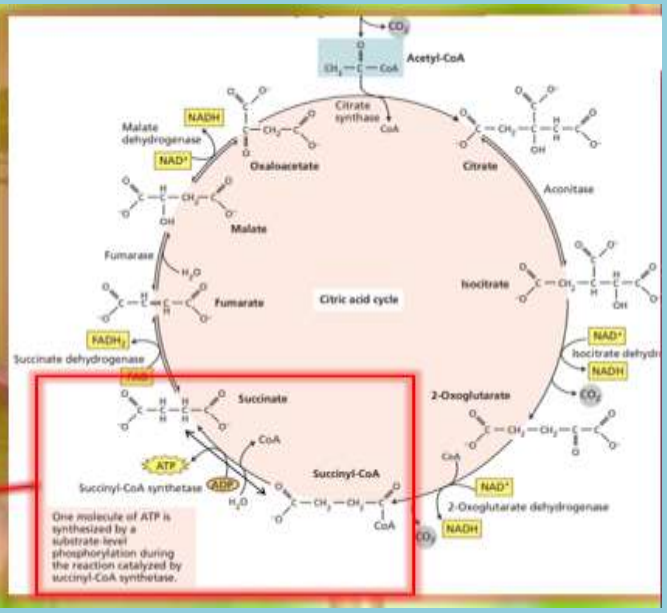



## STEP 5-CONVERSION OF SUCCINYL-COA TO SUCCINATE

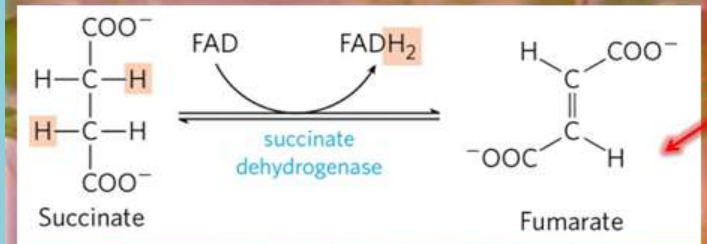
- **Substrate:-** Succinyl-CoA
- **Product:-** Succinate, ATP, CoA-SH
- **Enzyme:** Succinyl-CoA Synthethase
- **Cofactor:** ADP+Pi
- **Type of Reaction:** Substrate level Phosporylation(Reversible Reaction)

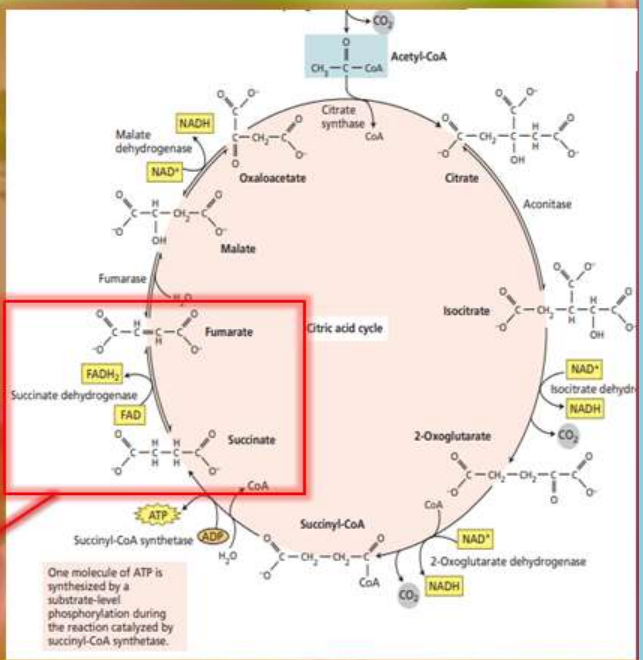



## STEP 6- OXIDATION OF SUCCINATE TO FUMARATE

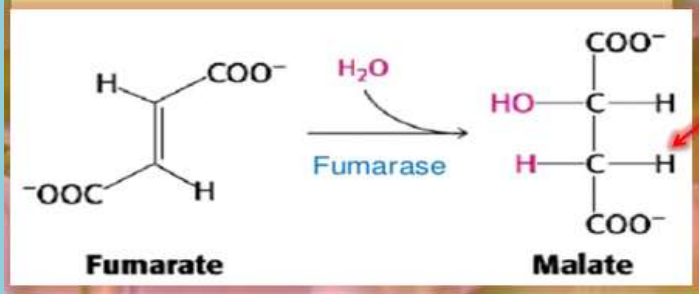
- **Substrate:-** Succinate
- **Product:-** Fumarate, $FADH_2$
- **Enzyme:** Succinate dehydrogenase
- **Cofactor:** FAD
- **Type of Reaction:** Dehydrogenation (Reversible Reaction)

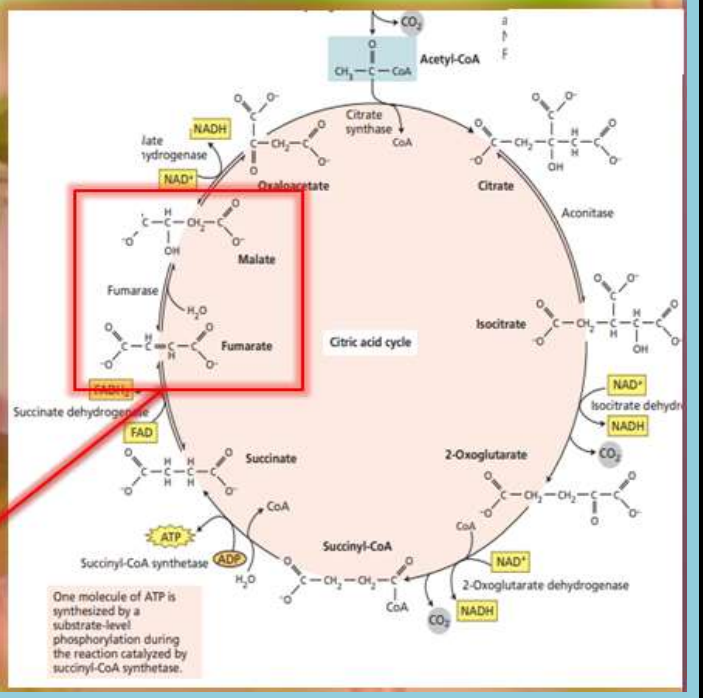


## STEP 7- HYDRATION OF FUMARATE TO MALATE

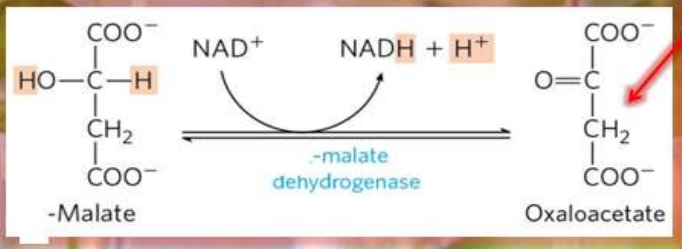
- **Substrate:-** Fumarate,water
- **Product:-** Malate
- **Enzyme:** Fumarase
- **Type of Reaction:** Hydration (Reversible Reaction)

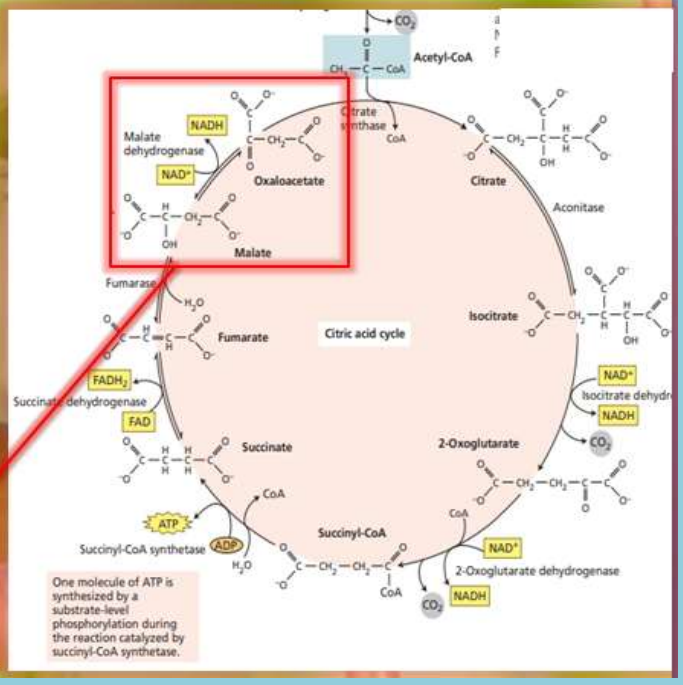



## STEP 8- OXIDATION OF MALATE TO OXALOACETATE

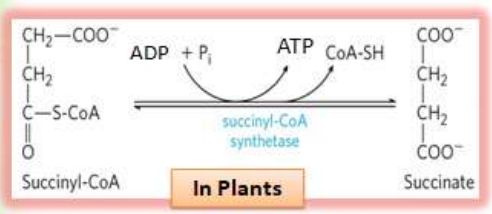
- **Substrate:-** Malate
- **Product:-** Oxaloacetate,NADH
- **Enzyme:** Malate dehydrogenase
- **Cofactor:** $NAD^+$
- **Type of Reaction:** Dehydrogenation (Reversible Reaction)

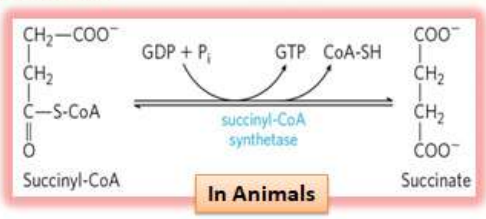



## UNIQUE FEATURES OF PLANT CITRIC ACID CYCLE

➢The step catalyzed by succinyl-CoA synthetase produces **ATP(Adenosine triphosphate) in plants** and **GTP(Guanosine triphosphate) in animals.**




➢A feature of the plant citric acid cycle is the presence of **malic enzyme** in the mitochondrial matrix of plants. This enzyme catalyzes the oxidative decarboxylation of malate. For degradation, malate is transported into the mitochondrial matrix, where malic enzyme can

oxidize it to pyruvate.

**Malate + NAD+ → Pyruvate + $CO_2$ + NADH**

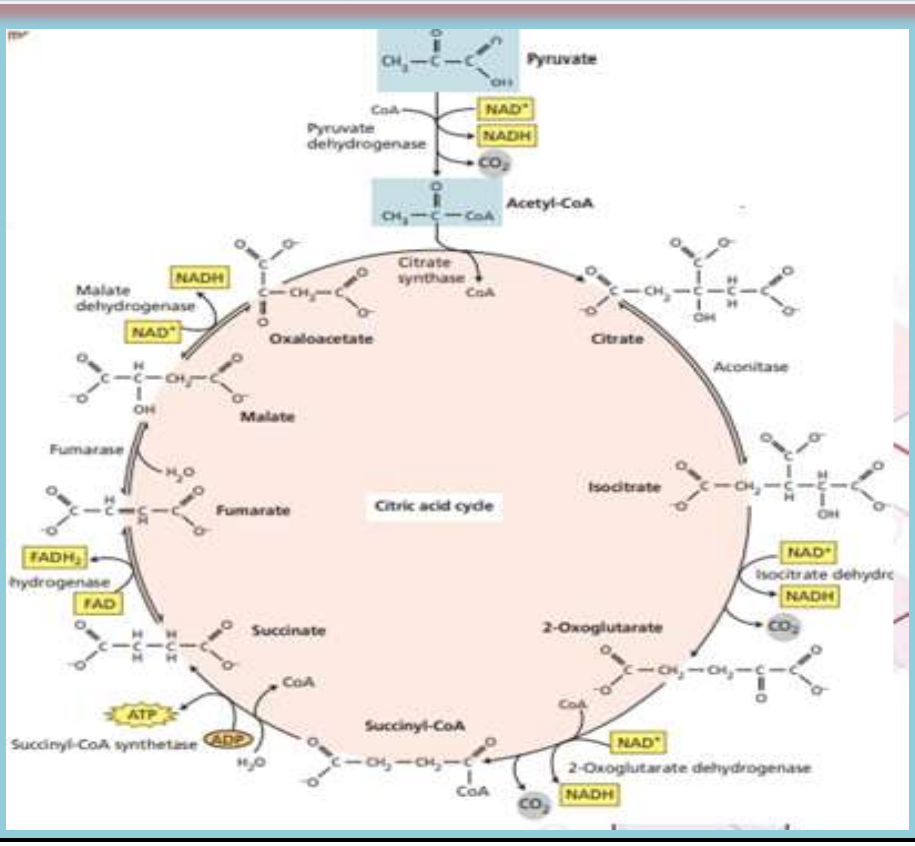
➢ **Gamma-aminobutyric acid** (GABA) is an amino acid that accumulates under several biotic and abiotic stress conditions in plants. GABA is synthesized from 2-oxoglutarate and degraded into succinate by the so-called **GABA shunt**, which bypasses the citric acid cycle enzymes.

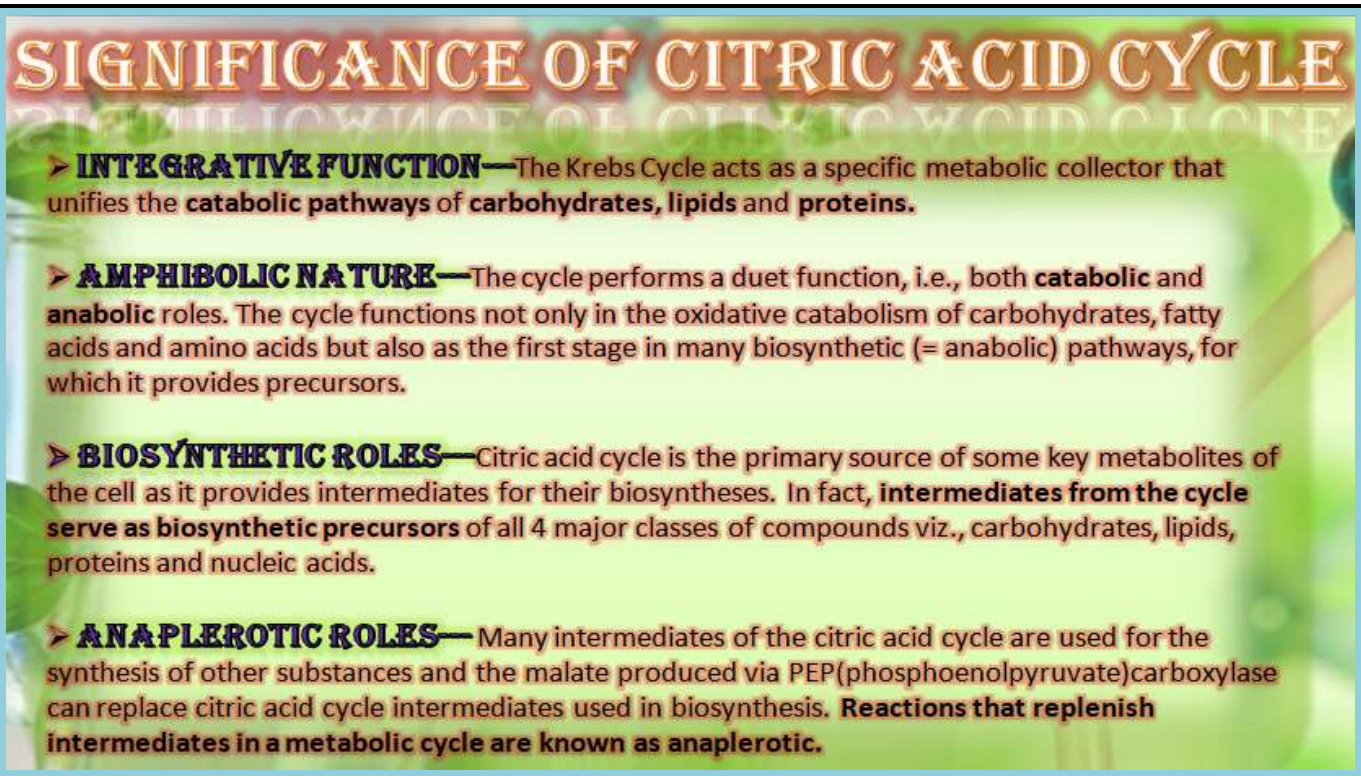
# STOICHIOMETRY OF THE PLANT CITRIC ACID CYCLE

➢ The stepwise oxidation of **1 molecule of pyruvate** in the mitochondrion gives rise to **3 molecules of $CO_2$, 4 NADH** and **1 $FADH_2$**. In addition, **1 molecule of ATP** is produced by a substrate-level phosphorylation.

➢ Or we can say **12 $CO_2$, 16 NADH, 4 $FADH_2$** and **4 ATP** have been produced for **each molecule of sucrose** oxidized.

| Metabolic pathway | Substrates | Products |
|---|---|---|
| **Citric acid cycle** | | |
| | 4 Pyruvate | 12 $CO_2$ |
| | 4 ADP + 4 $P_i$ | 4 ATP |
| | 16 $NAD^+$ (mitochondrial) | 16 NADH (mitochondrial) |
| | 4 FAD | 4 $FADH_2$ |






# Conclusion

In the past two decades, mitochondrial biology has undergone a renaissance partly due to the appreciation that mitochondria have important biological functions beyond ATP and macromolecules production. Indeed, mitochondria have evolved from passive to active players in determining cell fate and function. Mechanistically, TCA cycle metabolites have been demonstrated to control transcription factors and chromatin modifications to change cell function and fate. However, in many contexts the molecular details of how changes in TCA cycle metabolites abundance affect the expression of specific genes remains to be elucidated. Future investigations might also discover additional mechanisms by which TCA cycle metabolites exert signaling functions beyond post-translational modifications. Emerging evidence indicates that beyond cell autonomous functions, TCA cycle metabolites control physiology through non-cell autonomous functions. Discovering systemic effects of metabolites and their role in communicating different parts of the body will be of much interest for

the field in the upcoming years. A fascinating development is the use of derivatives of TCA cycle metabolites to ameliorate inflammatory diseases in humans. We hope to see more of the recent findings of TCA cycle signaling effects being translated into the clinic. Going forward, we predict that TCA cycle metabolites will continue to shed new light on biology, physiology, and diseases.


# Acknowledgement

I bow with reverence and profound sense of gratitude in expressing my sincere appreciation, heartfelt indebtedness and best regard to Dr.Tripti Roy, Dr. Debjani Sinha Ray and all my respected teachers for giving me this golden opportunity to present my topic and their active guidance during this whole work.

I would like to convey my sincere regards and pronounced gratitude to my parents for their meticulous supervision, valuable suggestions and expert comments throughout my assignment.

I am thankful to all my friends for their valuable suggestions and cooperation in carrying out the work.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# Paper-XIV

# SOIL-PLANT-ATMOSPHERE CONTINUUM CONCEPT

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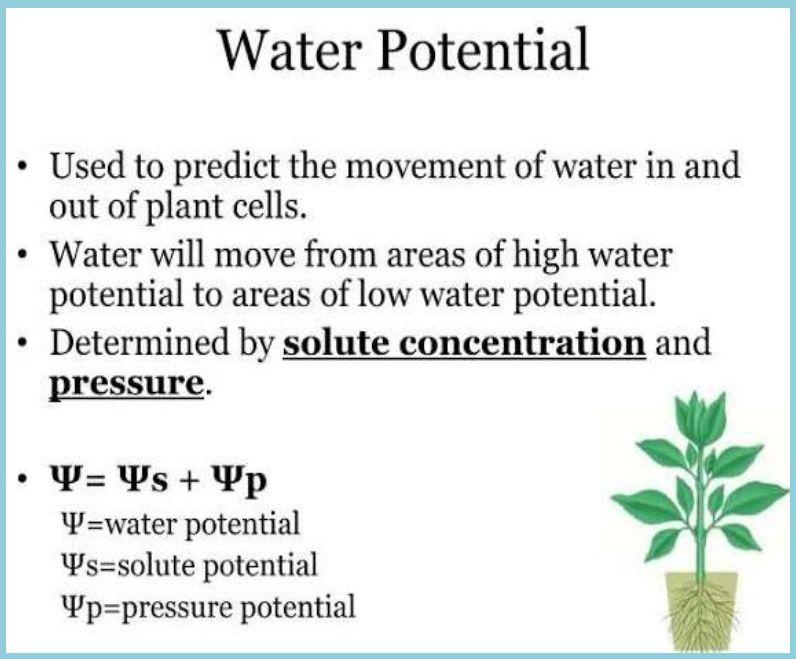
# Abstract

The soil-plant-atmosphere continuum (SPAC) is the pathway for water moving from soil through plants to the atmosphere. Continuum in the description highlights the continuous nature of water connection through the pathway.The low water potential of the atmosphere, and relatively higher (i.e. less negative) water potential inside leaves, leads to a diffusion gradient across the stomatal pores of leaves, drawing water out of the leaves as vapour followed by some stages :

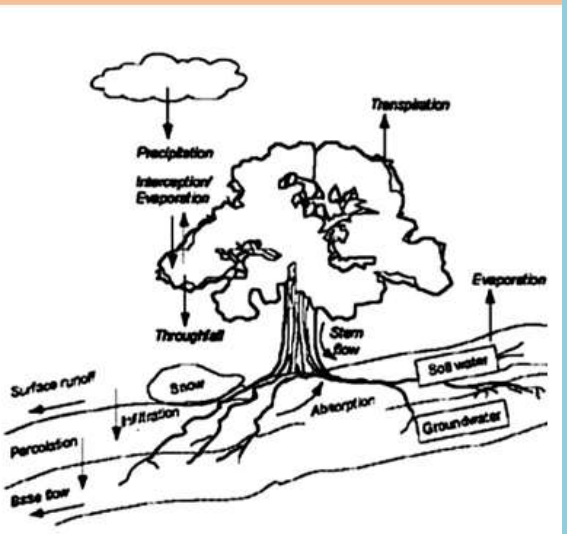
1.Flow of water in SPAC,
2. Flow of water in soil,
3. Uptake of water by root ,
4. Flow of water from roots to leaves ,
5. Evapotranspiration from the earth surface to the atmosphere ,
6. Magnitude difference in water potential and
7. Some factors controlling SPAC system (like soil particles, surface tension, diffusion **etc).**

# Introduction



## Overview of Soil-Plant Atmosphere Continuum (SPAC)

- All components of field environment such as soil, plant and atmosphere , form a **physically unified and dynamic system.**
- This unified system is called as **Soil-Plant-Atmosphere-Continuum or SPAC** in which, various water flow processes occur independently.
- Within SPAC , water moves from regions of **higher total water potential to the regions of lower water potential** . This rate depends upon hydraulic resistance of the medium.




# FLOW OF WATER IN SPAC

- Flow of water in SPAC is inversely proportional to the hydraulic resistance.

$$q = -\frac{\Delta\psi_1}{R_1} = -\frac{\Delta\psi_2}{R_2} = -\frac{\Delta\psi_3}{R_3} = -\frac{\Delta\psi_4}{R_4}$$

- This equation represents steady state flow where q is water flux (e.g. In m/day ) and the numerator values different pathways in the system.
- With R1 Representing soil to root pathway, R2 is root to xylem , R3 represents water movement from xylem to leaves and R4 represents water flux from leaves to atmosphere.

# FLOW OF WATER IN SOIL

- It is useful to think of water movement as the product of a driving force causing water to move and a factor representing the ease with which water moves in the soil.
- This was formalized by Henry Darcy in 1856 as

$$q = -K\frac{dTH}{dx}$$

• Where q is the volume of water flowing through a unit cross-sectional area of soil per unit time.
• K is the saturated hydraulic conductivity of the soil.
• TH is the total hydraulic head and x is the position coordinate in the direction of flow. This equation is known as **Darcy's law.**
• For uniform saturated soils, it is useful to write this equation as

$$q = K \frac{TH_A - TH_B}{L_{AB}}$$

Where **THA** is the total head at the inlet end of the soil, **THB** is the total head at the outlet end of the soil column and **LAB** is the distance between the inlet and outlet.

**The hydraulic conductivity** , **K**, represents ease with which water flows through a soil. Its value depends upon soil properties and properties of soil water.

## UPTAKE OF WATER BY ROOT

Uptake of water by roots depends on number of factors such as soil hydraulic conductivity, rooting depth and density, root distribution soil osmotic potential, depth of water table and evaporative demand of atmosphere. In field soil, root length density in the topsoil is usually so high that the local rate of uptake of water is never likely to be limited by soil properties. However, in subsoil, roots become sparse and water flow through soil might limit uptake rates.

• A substantial resistance to water uptake exists at interface between soil and root, known as interfacial resistance.
• the interfacial boundary is only a few hundred micrometers thick and rich in organic substances secreted by the root to form a rhizosphere.
• Two properties of an interfacial zone could influence water flow into roots.

First, exclusion of ions at root membranes might result in a large build up of these ions outside membranes. High osmotic pressures outside roots impede uptake of water.

Second, possible impediment to water flow from soil to root is that physical gaps might form at soil-root interface,either through roots growing into pores that are much wider than root axis or because of roots shrinking within a pore into which they once fitted snugly.

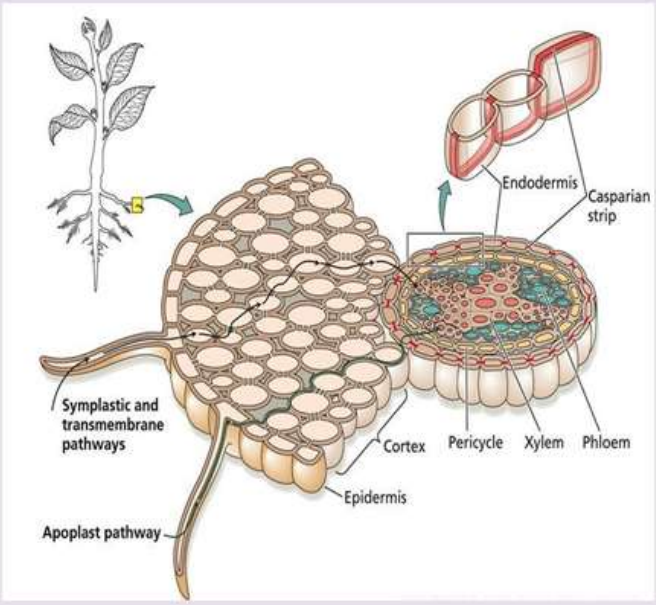
## WATER FLOW INSIDE ROOT

• The function of plant roots is dual : they anchor the plant to the soil matrix and they absorb water and nutrients from the soil .

• Water and nutrients preferentially enter the root through small root hairs that are located on the root epidermal cells.

• water travels this single cell epidermal layer to reach the cortex.

• In the cortex , water travels along two pathways:
a) it moves through the **apoplast**
b) it moves through the **symplast and transmembrane pathway.**




## WATER FLOWS FROM ROOT TO LEAVES

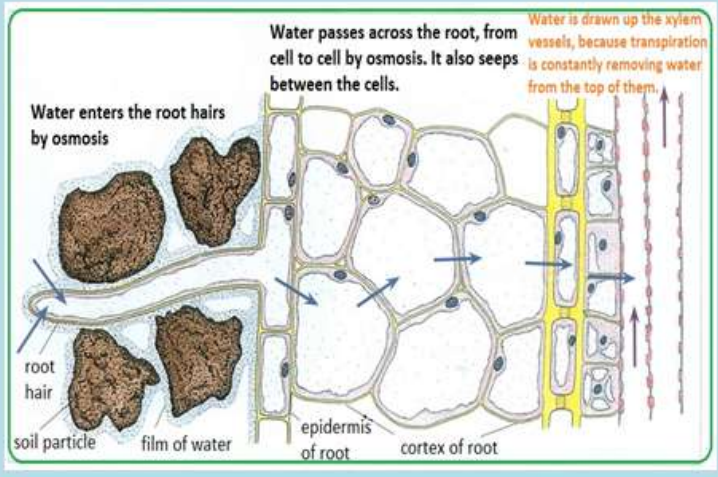
• The water conducting elements from vascular tissue are primarily the non-living and **heavily thickened and lignified single-celled tracheids and multicellular xylem vessels,** provide a low resistance pathway for the transport of water.

• They act as a network of tubes connected one to another.

• Pressure-driven **bulk flow** moves water long distances through the xylem.

• From these **xylem tubes** , water can ascend from the root up to the small veins in the **leaves.**

• **Cavitation(leading to the formation of small vapour filled cavities or bubbles in the liquid)** breaks the water column and prevents the transport of water under tension.

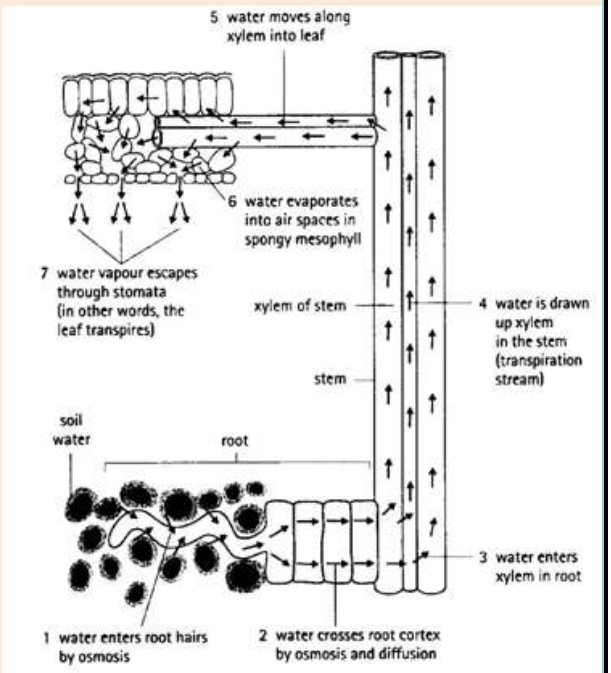


## WATER FLOWS FROM ROOT TO LEAVES

• At vein ends , water is released from xylem.

• After crossing mesophyll cells , it reaches substomatal cavity from where it diffuses through stomatal pores into atmosphere **as water vapour.**

• The ascent of liquid water through the tree induced by this transpiration process is called as **sap flow (transpration stream)**



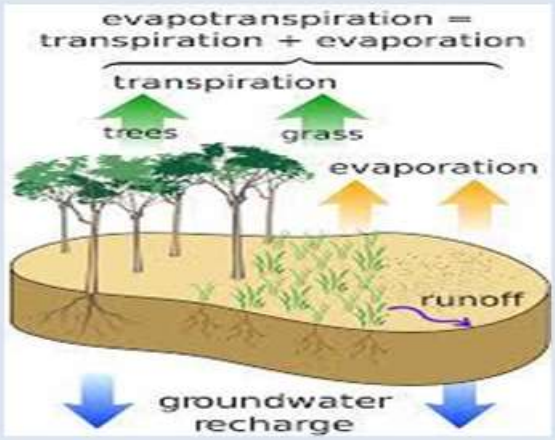

## EVAPOTRANSPIRATION AND WATER CONSUMPTION

• **Evapotranspiration** is some of evaporation and **transpiration** from the earth's surface to the atmosphere.

• Because evaporation and transpiration are practically difficult to separate.

• **Evaporation accounts for movement of water to air from soil surface, plant canopies and water bodies.**

• **Transpiration accounts for the water movement within plant and subsequent loss through water vapour through stomata in its leaves.**




For SPAC **THREE** basic physical conditions to be met

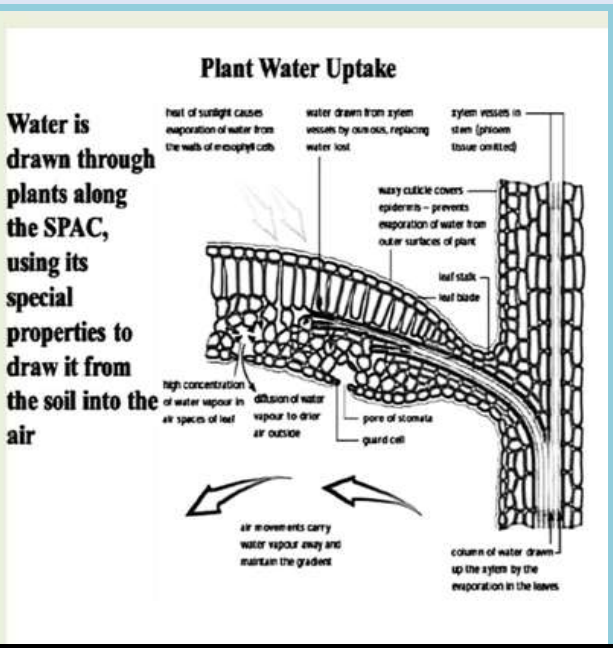
• In the soil and the xylem, liquid water moves by bulk flow or mass flow in response to a pressure gradient.

• When liquid water is transported across the membrane, the **driving force** is **the water potential difference across the membrane.**

• A) Continuous heat supply to change liquid water into **vapour.**

• B**) Vapour gradient** must be present to maintain flux. Vapour must be trsansported away **by diffusion or convection.**

• C) Continuous water supply which depends on the content and **water potential** in plant body as well as on its **conductive properties.**



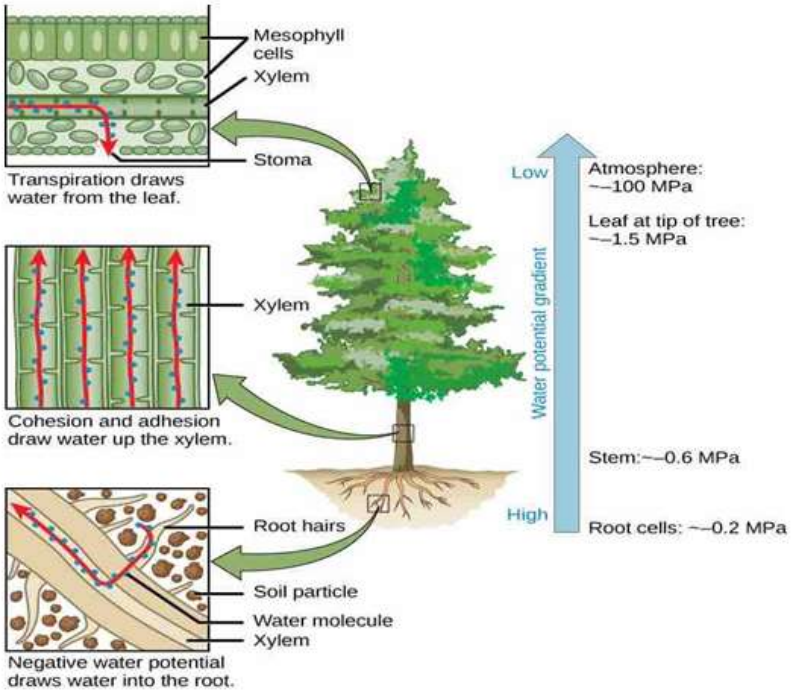
The key element in the transport of water from the soil to the leaves is the generation of **negative pressure within the xylem** due to **capillary forces** within the cell walls of transpiring leaves.

❑ At the other end of the plant **soil water** is also held by **capillary forces.**

❑ This results in a "**tug- of -war**" on a rope of water by **capillary forces** at both the ends.

❑ As a leaf loses water due to **transpiration,** water moves up the plant and out of the soil driven by **physical forces**, without the involvement of **any metabolic pump.**

❑ **The energy** for the movement of water is ultimately supplied by **the sun.**




## MAGNITUDE IN WATER POTENTIAL

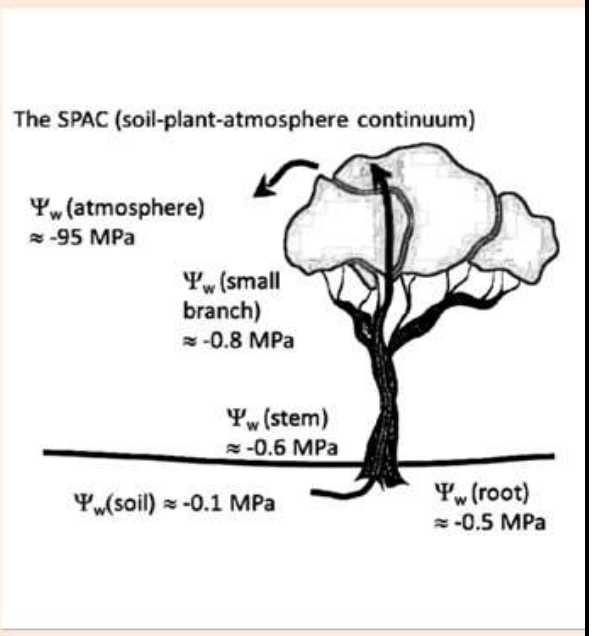
•The **total potential difference** between soil moisture and atmospheric humidity range from tens of MPa and in arid climates can exceed 100 MPa.

• The potential drop is the soil towards the roots varies from less than 100 Kpa .

• In roots, potential drop is somewhat greater than from soil to roots.

• The major portion of **potential drop** occurs between **leaves** and **the atmosphere.**

This fig shows **the net movement of water down its potential energy gradient ,from highest water potential in the soil to lowest water potential in the air.**




# FACTORS CONTROLLING SPAC SYSTEM

SPAC is determined by several factors like

- Soil particles and surface tension
- Water holding capacity and soil stabilization
- Relative humidity
- DPD( Diffusion Pressure Deficit) and OP(Osmotic Pressure) of plant cell

## ➢ 1) Soil Particles and Surface Tension

**Soil texture**:

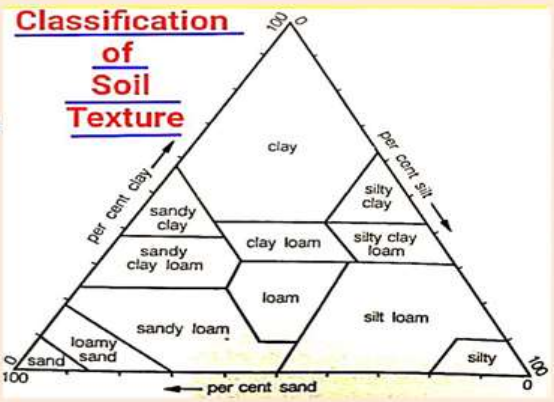
• It is the composition of soil in terms of the proportion of small, medium and large soil particles (clay, silt, and sand, respectively) in a specific soil mass.

•The **soil texture** depends on **these particles.**

**Soil structure**:

• The arrangement of soil particles into stable units called **aggregates**, which give the structure of soil.

• Aggregates can be loose or miscible, or they can form distinct, uniform patterns.




| Soil Texture | Soil Particles |
|---|---|
| 1. coarse soil | sand or loamy sand |
| 2. medium soil | loam, silt loam or silt |
| 3. fine soil | sandy clay, silty clay, or clay |

| Name of the Soil Particle | Diameter (mm) |
|---|---|
| Gravel | ≤2 |
| Coarse Sand | 2- 0.2 |
| Fine Sand | 0.2-0.02 |
| Silt | 0.02-0.002 |
| Clay | ≥0.002 |

**Soil porosity**:

• It is the **space between soil particles**, which contains variable amounts of water and air.

• water can be held **tighter in small pores than in large ones, so fine soils are capable of holding more water than the coarse soils.**

**WATER INFILTRATION:**

• It is the movement of water from the soil surface into the soil profile.

• Wide pore spacing at the soil surface increases the rate of water infiltration, so **coarse soils** have a **higher infiltration than fine soils.**

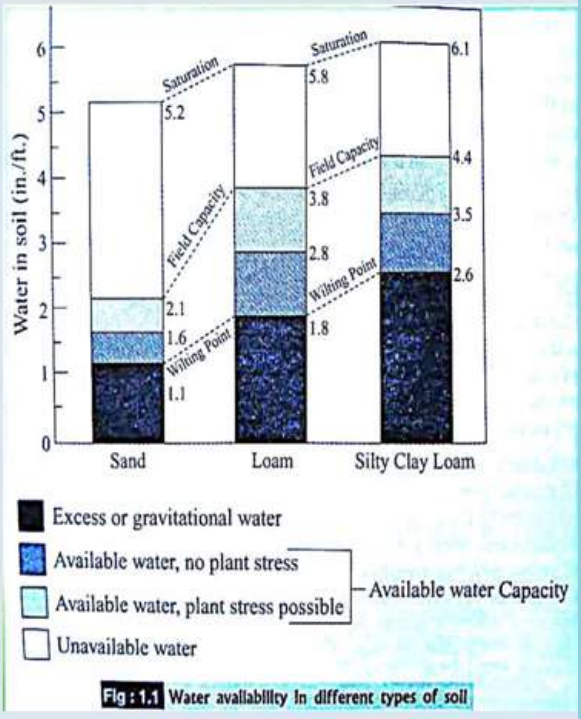



Fig : 1.1 Water availability in different types of soil

| Type of Soil | Size of Soil Particles | Soil Porosity ( Size & Number of Pores in Soil ) | Water holding Capacity |
|---|---|---|---|
| Fine Soil | Smaller Sized | Smaller sized but Greater number Pores | Larger Amount |
| Coarse Soil | Bigger Sized | Bigger Sized but Smaller number Pores | Smaller Amount |

**Soil Permeability:**

• It is **the movement of air and water through the soil.** It affects the supply of rhizosphere air, moisture, and nutrients available for plant uptake.

• It is determined by the relative rate of moisture and air movement through the most restrictive layer within the upper 40 inches of the effective root zone.

| Type of Soil | Soil Permeability |
|---|---|
| coarse soil | Water and air rapidly permeate |
| Fine Soil | Water and air slowly permeate |

**SURFACE TENSION**:

• The **cohesive forces between liquid molecules are responsible for the phenomenon known as surface tension.**

• water has 20 degree C has a surface tension of 72.8 dynes/cm compared to 22.3 for ethyl alcohol and 465 for murcury.

• **increase in surface tension reduces the permeability of water through soil particles.**

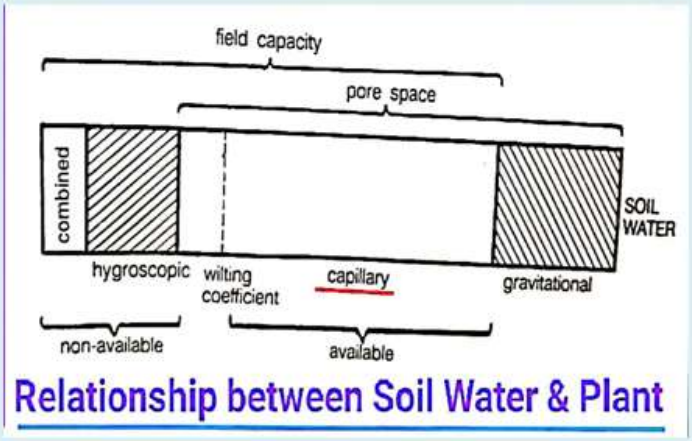
➢2) **Water holding capacity and soil stabilization**

• The total amount of water present in the soil, denotes its **field capacity** .
• **total amount of soil water = Gravitational water+ chemically combined water + capillary water + adsorbed water**
• The capillary water is available for the plant, it will represent the water holding capacity of the soil and the amount of water present in the soil when the plant shows wilting is called **wilting Co-efficient**
• Water holding capacity is controlled primarily by soil texture and organic ,matter.
• **When the organic matter percentage increases in the soil , the water holding capacity also increases because of the affinity of organic matter towards water.**

| Soil Texture Class | Available Water Holding Capacity ( Inches/ Foot of Depth of Soil ) |
|---|---|
| 1. Coarse Sand | 0.25 – 0.75 |
| 2. Fine Sand | 0.75 – 1.00 |
| 3. Loamy Sand | 1.10 – 1.20 |
| 4. Sandy Loam | 1.25 – 1.40 |
| 5. Fine Sandy Loam | 1.50 – 2.00 |
| 6. Silty Loam | 2.00 – 2.50 |
| 7. Silty clay Loam | 1.80 – 2.00 |
| 8. Silty Clay | 1.50 – 1.70 |
| 9. Clay | 1.20 – 1.50 |

• A soil with **a high percentage of silt and clay particles (i.e.fine soil) has a higher water holding capacity.**

• Water availability is determined by the **water holding capacity.**
• Soil with smaller particles (silt and clay) have a larger surface area than larger sand particles . **A large surface area allows a soil to hold more water.**
• Excess or gravitational water drains quickly from the soil after a heavy shower because of the pull by the **gravitational force.**
• Plants use small amount of this water before it moves out of the rhizosphere.



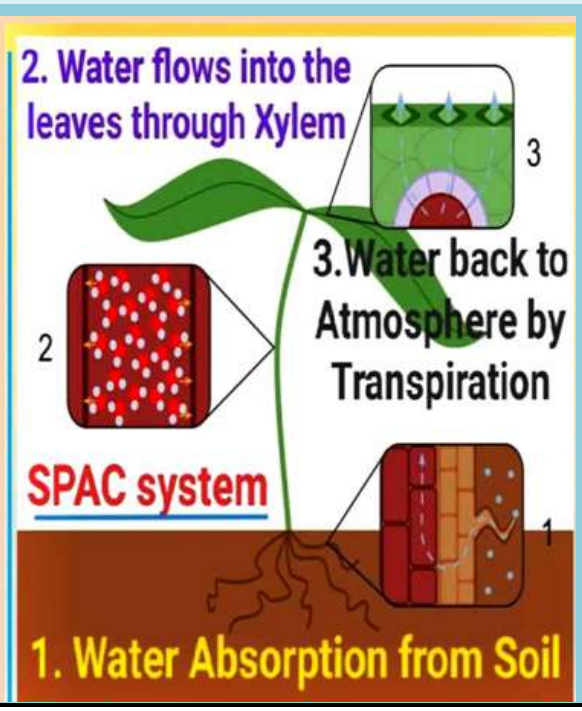
Relationship between Soil Water & Plant

• Available water is retained in the soil after the excess water has been **drained out(field capacity) . This water is the most important for crop or forage production.**

• plants can use approximately 50% of it without exhibiting stress,but if less than 50% is AVAILABLE ,drought stress may result.

• Unavailable water is the soil moisture that is held so tightly by the soil, that it cannot be extracted by the plant.

• Thus ,water may remain in the soil even below plant's **wilting point.**

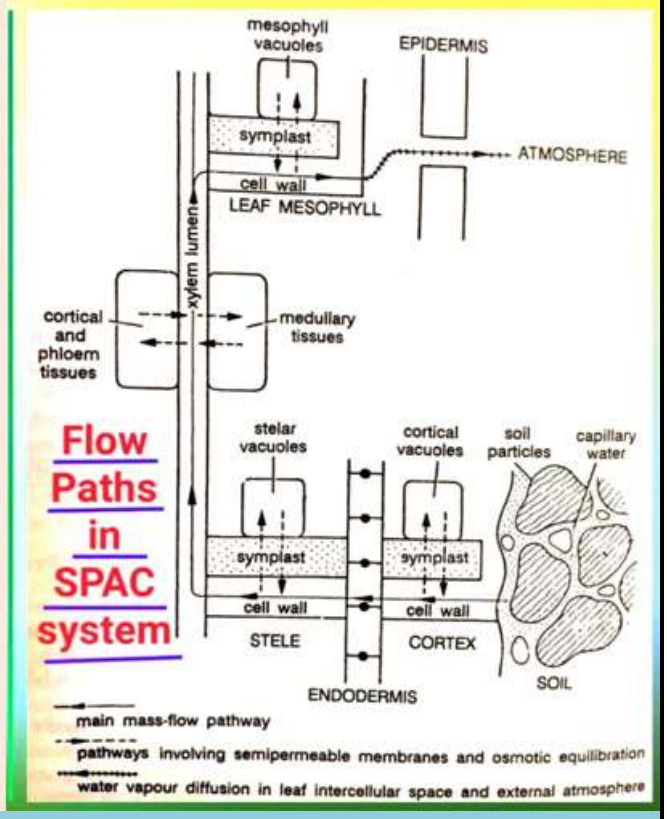


➢ **3) Relative humidity:**

• The amount of water vapour that exists in a gaseous mixture of air and water vapour is known as **Relative Humidity.**

• The relative humidity will drop by a factor of 2 for each 20 degree F or 10 degree C increase in temperature, assuming coservation of absolute moisture.

• **This will reduce the water content of the soil and will limit the water availability to the plant roots.**

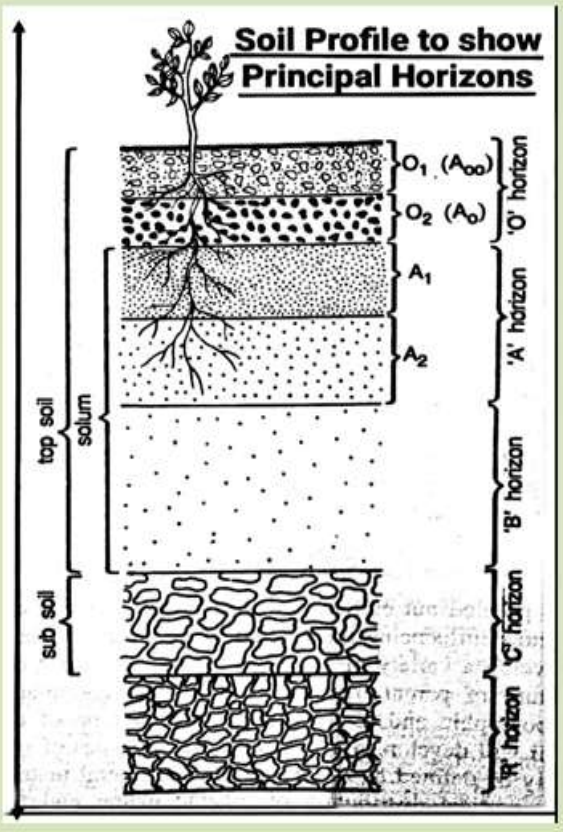



➢ **4) DPD (Diffusion Pressure Deficit) and OP (Osmotic Pressure) of plant cell**

• The capillary water content of the soil is controlled by the osmotic potential and the increase in the solute potential reduce the water absorption ability.

• the water extraction rate by the plant roots from the soil is not constant and follows a diurnal cycle; usually it is relatively low in the early morning hours but increases gradually till afternoon.

• **the water extraction rate depends on the size of the plant , the density of the root, the resistance faced by the water molecules to enter the plant roots and the evaporative conditions prevailing in that place.**



# Conclusion

The SPAC plays an important role in the movement of water from the soil, through the plant and to the atmosphere along an interconnected film of liquid water. The SPAC integrates all components (soil, plant, animals, and the surrounding atmosphere) into a dynamic system in which the various transport processes involving energy and matter occur simultaneously. The SPAC is fundamental to the hydrologic cycle, the ability of plants to photosynthesize, and therefore to most life on earth. Understanding the SPAC is crucial in plant physiology studies.

# Acknowledgement

I would like to express my gratitude to Dr.Tripti Roy and also Dr.Debjani Sinha Roy, associate professors, Dept. of Botany, Bethune college, Kolkata for giving me the opportunity to present this topic. It has been a learning experience for me. Thank you ma'am for your constant support and guidance. I would also like to give my Thanks to my beloved classmates for listening to the presentation.

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# Paper-XV

# Rhizobial Infection and Nodule Organogenesis In Legumes

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# Abstract

Rhizobia are a "group of soil bacteria that infect the roots of legumes to form root nodules". Rhizobia are found in the soil and after infection, produce nodules in the legume where they fix nitrogen gas (N2) from the atmosphere turning it into a more readily useful form of nitrogen. The N(2)-fixing nodules elicited by rhizobia on legume roots represent a useful model for studying plant responses to nitrogen fixing bacteria. Nodule formation implies a complex progression of temporally and spatially regulated events of cell differentiation/dedifferentiation involving several root tissues. Here, in this presentation I have described the different Stages of Infection and Nodule Organogenesis and the Genes governing it.

# Introduction

## RHIZOBIA-LEGUME ASSOCIATION

✓Symbiotic association

✓Of *great ecological significance*

✓Data shows typically a hectare of legume-rhizobium association is responsible for fixing *25-60 kgs of dinitrogen* annually

✓So, extensively studied : earlier from a morphological point of view, more recently from a molecular/biochemical and genetic point of view

✓Microbiont belong to 3 genera : *Rhizobium*, *Azorhizobium*, *Bradyrhizobium*

✓Legume may be Sesbania, Glycine, Medicago, Lens etc.

✓Rhizobia-legume symbiosis begins by *Rhizobial Infection + Nodule Organogenesis*

Soybean root with abundant nodules, June 30, 2015.

White clover root with nodules

## RHIZOBIAL INFECTION AND NODULE ORGANOGENESIS IN LEGUMES:

▶ Achieved by a *sequence of multiple interactions* between the microbiont (rhizobium) and the host (legume plant)

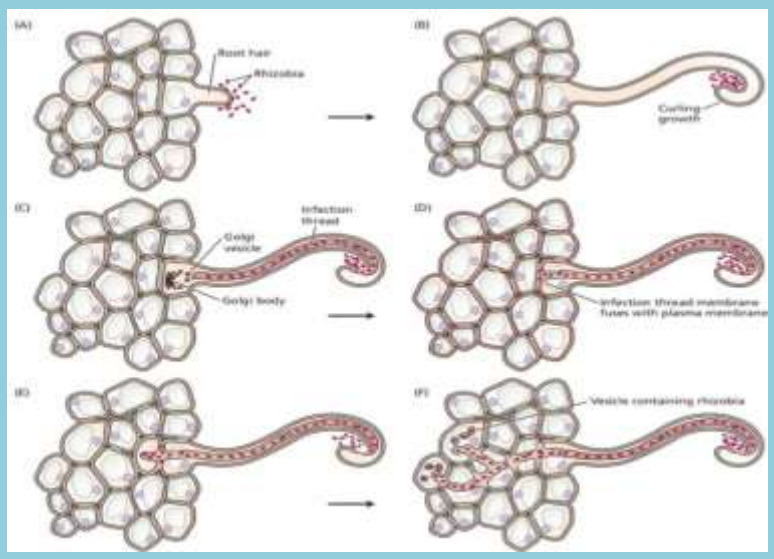
▶ Starts with a *chemical dialogue* mediated by the chemicals present on the surface of both host and symbiont, only on the successful completion of which the infection takes place

▶ So, *Specificity* plays a role- some rhizobia can infect only a single host plant while some can infect a range of hosts

▶ As many as 9-10 developmental stages, can be broadly divided into 4:

1. *Multiplication* of the rhizobia, *colonization* of the rhizosphere, and *attachment* to epidermal and root hair cells
2. *Nodule initiation* and *development* in the root cortex
3. Characteristic *curling* of the root hair and *invasion* of the bacteria to form an *infection thread*
4. *Release* of the bacteria from the infection thread and their *differentiation* as specialized nitrogen-**FIXING CELLS**

# STAGES OF INFECTION AND NODULE ORGANOGENESIS




## STAGE I: MULTIPLICATION OF THE RHIZOBIA, COLONIZATION OF THE RHIZOSPHERE, AND ATTACHMENT TO EPIDERMAL AND ROOT HAIR CELLS

- Host roots secrete *chemoattractants* like (iso)flavonoids, betaines
- Rhizobia move by *+ve Chemotaxis*
- *Colonization* of rhizosphere
- Bacteria secrete *host specific morphogenic signal molecules* (*Nod Factors/Nodulation Factors*)
- Nod Factors = lipochitooligosaccharides
- Bacteria attach to the lectins of the host

## NOD FACTOR RECOGNITION IN HOST PLANT

- Nod factor receptors = Proteins with intracellular *kinase* domains and extracellular *Lys M* domains
- Nod factor binds to the receptors
- Activation of Lys M domains

•*Rhizobium nod factor signal* transduction pathway begins

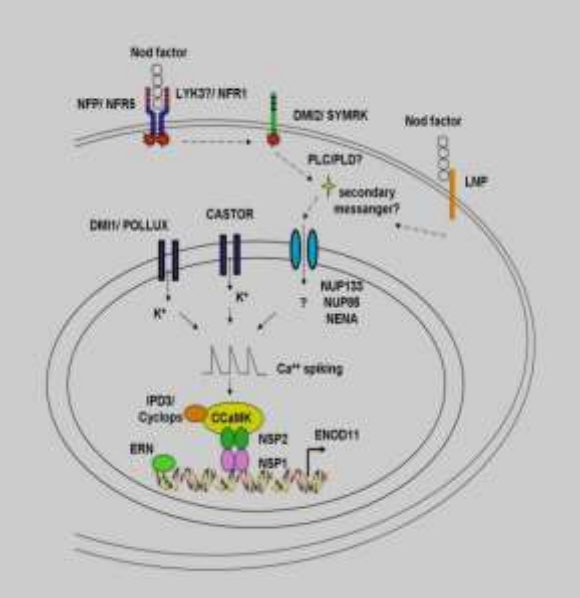
•Activation of *trimeric G-proteins* coupled with rapid influx of calcium ions

•Cytosolic $Ca^{2+}$ conc. oscillates around the nucleus (Calcium Spiking)

•Oscillations recognized by *calcium ion/ calmodulin dependent protein kinases* (CaMK) associated with *CYCLOPS* protein

•*Nod factors responsive transcriptional regulators* associate with the promoters of *Nod factor-inducible genes*

•Thus, phenotypic changes like root hair deformation begin in response to nod factors




**Cascade of reactions after binding of nod factor to nod factor receptor R showing calcium efflux and trimeric G-protein**

## STAGE II: NODULE INITIATION AND DEVELOPMENT IN THE ROOT CORTEX

❑ Coincides with the first step

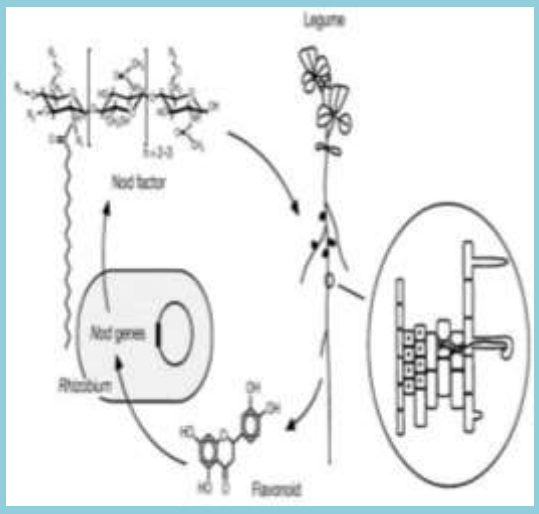
❑ Nod factors result in an increase in the production of *root hair with tip curled*, growth of *shorter and thicker roots* with *more branching*

❑ Mitogenic signals released by rhizobia stimulate localized cell divisions in the root cortex forming ***Primary Nodule Meristem***

❑ Cells of *pericycle* also divide

❑ Both dividing masses fuse to form a specialized structure called the *nodule*

❑ Nodule keeps increasing in size due to the activity of the nodule meristem




**Molecular interactions between a legume root and *Rhizobium***

## STAGE III: CHARACTERISTIC CURLING OF THE ROOT HAIR AND INVASION OF THE BACTERIA TO FORM AN INFECTION THREAD

▶ Colonies of rhizobia *get entrapped* by the curled growing tip of root hair (*infection pocket*)

▶ Bacteria secrete enzymes like cellulase, pectinase, hemicellulase to *digest the cell wall* materials

▶ Root *tip ceases growth*

▶ Bacteria *enter the plasma membrane* of the cell

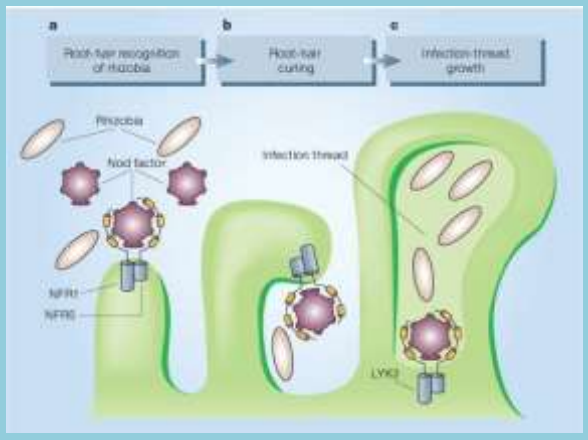
▶ *Cell membrane invaginates* inwards forming a tubular intrusion called *infection thread*. The infection thread contains the rhizobia

▶ *Vesicles* derived from the Golgi apparatus *fuse with the infection thread*- Elongation of infection thread

▶ *Deposition of* thin layer of *cellulose* over inner surface of infection thread

▶ Bacteria reach the base of the root cell. Infection *thread fuses with the plasma membrane*. Bacteria get *released into the apoplastic space*. Rhizobia again degrade the cell wall of the next cell and *infection continues*

▶ *EPS* and *lipopolysaccharide* on the outer surface of bacteria suppress the host plant's immune response




**Sequence of steps leading to formation of infection thread**

## *STAGE IV: RELEASE* OF THE BACTERIA FROM THE INFECTION THREAD AND THEIR *DIFFERENTIATION* AS SPECIALIZED NITROGEN-FIXING CELLS

❖ Rhizobia reaches the nodular cells and *branch out* so that many cells can be infected at a time

❖ Bacteria *enter the host cells* in the nodule by endocytosis

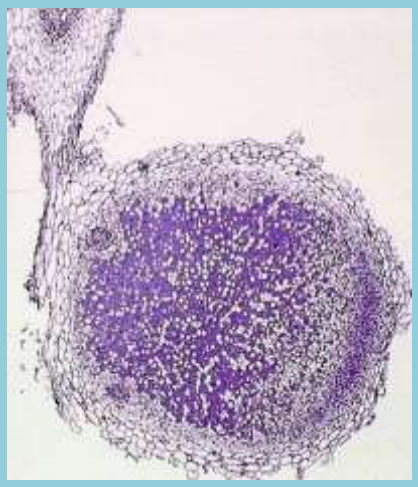
❖ The *infection thread buds* off

❖ Small vesicles containing one or more rhizobia are formed called symbiosomes

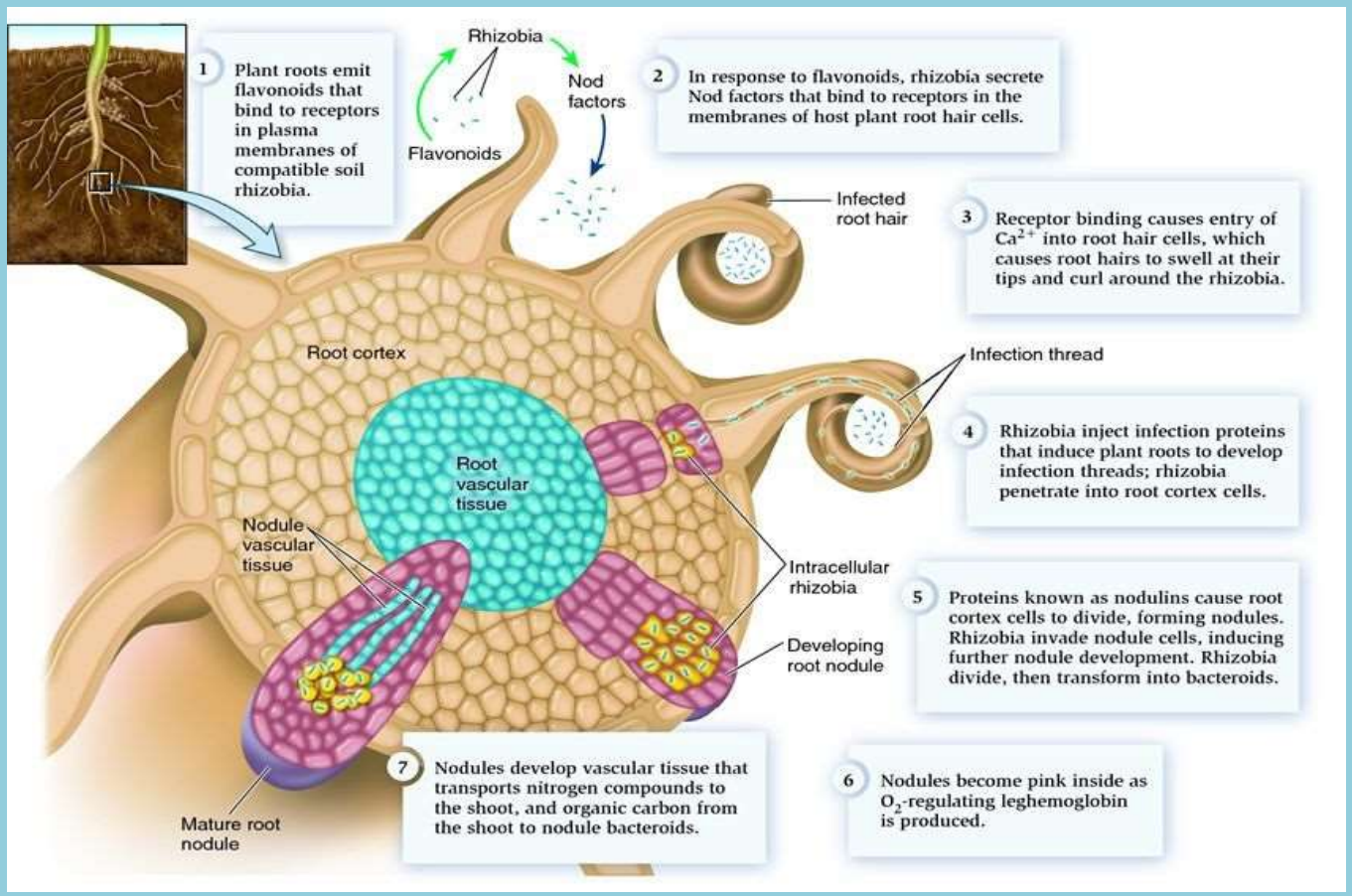
❖ *Bacterial division stops*. They enlarge and differentiate to form specialized, nitrogen fixing cells called bacteroids (terminally differentiated cells) with the membrane of the vesicle as peribacteroid membrane

❖ As nodule grows bigger, more and more cells get infected

❖ *Vascular connections are established* between the nodule and the main vascular system of the plant to supply carbon source to the nodule and to export fixed nitrogen from the nodule



**Root nodules sampled from ground beans after light microscopy**




**...At a glance...**

# GENES GOVERNING NODULE FORMATION

## NOD GENES

✓ Early stage of infection

✓Synthesize and regulate the synthesis of chitooligosaccharide core of the nod factors

✓Present in the Sym (Symbiosis) plasmid of rhizobia

✓Promoter of all the operons except the Nod D gene contains a highly conserved sequence called the nod box

✓*nod A gene*: N-acetyltransferase that catalyzes the addition of a fatty acyl chain

✓*nod B gene*: chitin-oligosaccharide deacetylase that removes the acetyl group from the terminal nonreducing sugar

✓*nod C gene*: chitin-oligosaccharide synthase that links N-acetyl-D-glucosamine monomers

✓*nod D gene*: Its activated product *switches on the transcription of the other Nod genes* by binding to the nod box of the other genes

✓*nod E, F, G, H gene*: Host specific genes. These code for modifications in the nod factors

✓*nod L gene:* Add specific substitutions at the reducing or nonreducing sugar moieties of the chitin backbone, Thus help in host specificity

## nod genes (In host)

❑ These are found in host genome

❑ Form proteins called **nodulins**- *early nodulins* and *late nodulins*

❑ Early nodulins are expressed during infection process and nodule development. Involved with the infection thread plasma membrane and in the formation of nodule primordia

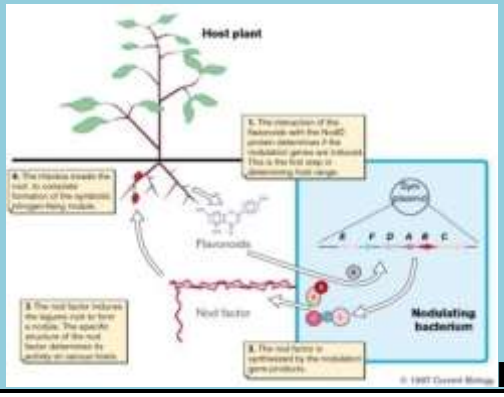



**Diagram depicting genetic control of nodulation**

# Conclusion

Rhizobial infection and nodule organogenesis in legumes is not only a great example of beneficial plant-microbe symbiosis (mutualism) in the natural world but is also a crucial step in the process of Biological Nitrogen Fixation by the legumes. Biological Nitrogen Fixation or BNF (discovered by Beijerinck in 1901) is the process of conversion of atmospheric dinitrogen to ammonia or other nitrogen containing compounds by biological agents called biological nitrogen fixers thus marking the entry point of molecular dinitrogen into the Biogeochemical Cycle. The process of Biological Nitrogen Fixation is an important part of the nitrogen cycle. It is an important supplier of N for plants including the crop plants thereby promoting sustainable agriculture as it decreases the need for exogenous fertilizer.

# Acknowledgement

I would like to express my gratitude to Dr. Tripti Roy, Associate Professor, Dept. of Botany, Bethune College, Kolkata for giving me the opportunity to present this topic. It has been a learning experience for me. Thank you ma'am for your constant support, guidance and mentorship.

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# Paper-XVI

# PHARMACOGNOSY-A SCIENCE OF DRUG AND KNOWLEDGE

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# Abstract

Pharmacognosy is the study of the physical, chemical, biochemical and biological properties of drugs, or drug substances of natural origin as well as the search for new drugs from natural sources. It has played an important role in the development of pure science and also in various purposes.

The drugs are classified through five systems mainly, i.e.,

1. Chemical Classification,
2. Pharmacological Classification,
3. Morphological Classification,
4. Taxonomical Classification,
5. Alphabetical Classification.

Drugs are evaluated to identify and to determine its quality and purity. The methods are:

1. Macroscopic Evaluation,
2. Microscopic Evaluation,
3. Chemical evaluation,
4. Physical evaluation,
5. Biological evaluation.

# Introduction

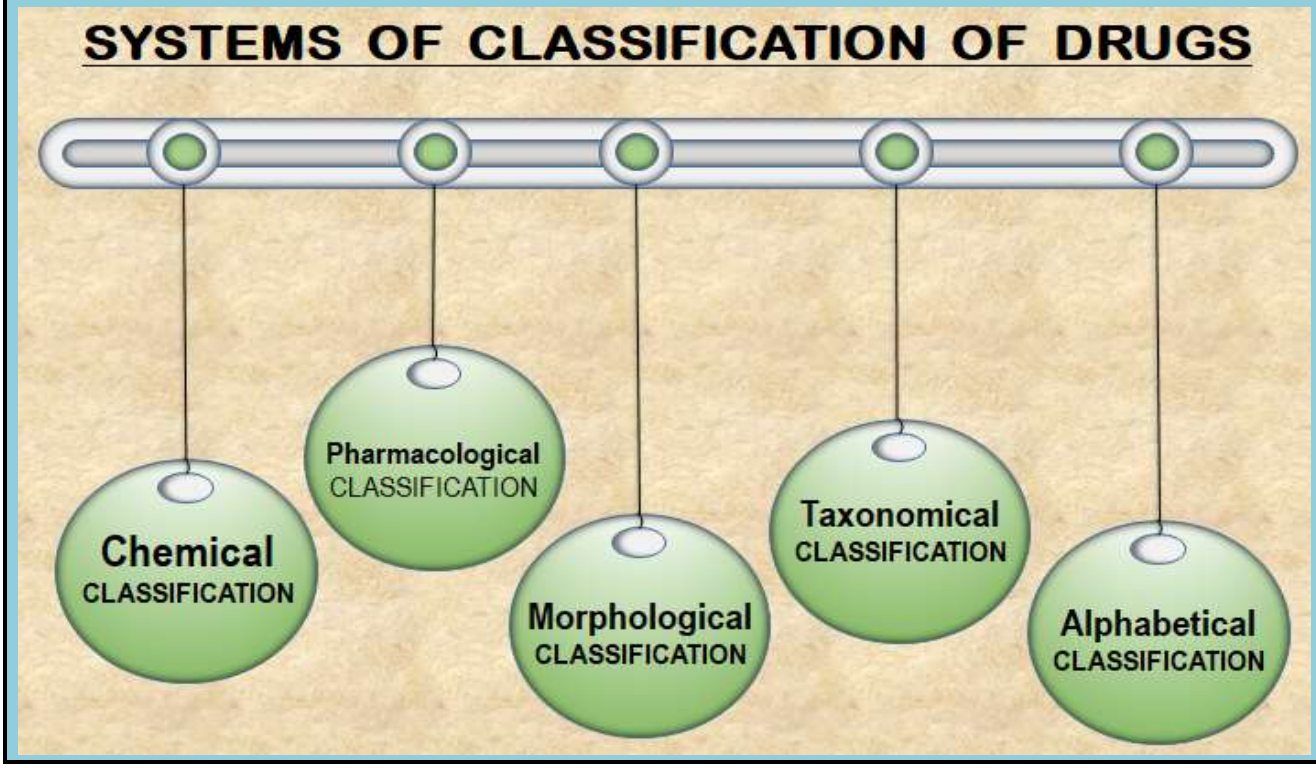
The word **'pharmacognosy'** is a combination of the Greek words **'pharmakon'** and **'gnosis',** i.e., **'drug'** and **'knowledge'**.

The American Society of Pharmacognosy defines it as **"the study of the physical, chemical, biochemical and biological properties of drugs, or drug substances of natural origin as well as the search for new drugs from natural sources".**

**Crude Drugs** are those that consists of natural substances like vegetable saps, extracts, secretions, etc. that have undergone only the process of collection and drying. They are used as such as therapeutic agents and their active constituents extracted by various means.

# IMPORTANCE OF PHARMACOGNOSY

a) It has played an important role in the development of pure science, e.g. descriptive botany, plant taxonomy and phytochemistry.
b) Study of plants used in medicinal, narcotic and other purpose by primitive tribes.
c) Screening of plants for their possible pharmacological value, particularly for their anti inflammatory, hypertensive, cytotoxic, antibiotic and anti-Parkinsonism.
d) To look for raw materials in the production of oral contraceptives, allergens, herbicides and insecticides.
e) To know about the active constituent when devising processes of extraction for the manufacture of chemicals or when studying compatibility in dispensing practice.
f) To evaluate drug based on physical characters and constituents.
g) To study the constituents and investigate their chemical reactions.



## SYSTEMS OF CLASSIFICATION (in brief)

- Chemical Classification: In this system the crude drugs are divided into different groups **according to the chemical nature of their identical constituents.**
- Pharmacological Classification: In this system drugs are grouped together **according to the therapeutic activity of their main chemical constituents.**
- Morphological Classification: In this system the crude drugs are classified **according to their morphological characters** like leaves, barks, seeds, fruits, roots, flowers etc.
- Taxonomical Classification: In this system the crude drugs are classified into **Phylum, Order, Family, Genus and Species.**
- Alphabetical Classification: In this system the crude drugs are **arranged in alphabetical order.**

# AN OUTLINE OF CHEMICAL CLASSIFICATION OF CRUDE DRUGS

• Carbohydrates – Carbohydrates are **polyhydroxy aldehydes** or **ketones** containing an unbroken chain of carbon atoms. Example, Gums - Acacia, Mucilages - Plantago seed etc.

• Glycosides – Glycosides are compounds which upon hydrolysis give rise to one or more **sugars (glycone)** and **non-sugar (aglycone)**. Example, Anthraquinone Glycosides - Aloe, Isothiocyanate Glycosides – Mustard etc.

• Tannins – Tannins are complex organic, non-nitrogenous derivatives of **polyhydroxy benzoic acids**. Examples, Black catechu, Ashoka bark.

• Volatile oils – **Monoterpenes** and **sesquiterpenes** obtained from plants. Example, Cinnamon.

• Resins – Complex mixture of compounds like **resinols**, **resin acids**, **resinotannols**, **resenes**. Example, Podophyllum etc.

• Lipids – Example, Fixed oils – Castor, Waxes – Beeswax.

• Alkaloids – Nitrogenous substance of plant origin. Example, Quinoline – Cinchona

• Protein – Gelatin

• Vitamins – Yeast

• Triterpenes – Rasna

## Plant Sources Of Chemical Substances In Pharmacognosy (Chemical Classification)

Gum *(Acacia)*

Anthraquinone Glycosides *(Aloe)*

Tannin *(Black catechu)*

Volatile Oil *(Cinnamomum)*

Resin *(Podophyllum)*

Resin *(Ricinus)*

## An Outline Of Pharmacological Classification Of Crude Drugs

| Pharmacological Action | Drugs |
|---|---|
| Anticancer | Vinca |
| Anti-inflammatory | Colchicum |
| Antiasthmatic | Ephedra |
| Antispasmodic | Datura |
| Antiamoebic | Ipecac Roots |
| Analgesic | Opium |
| Carminatives | Peppermint |
| Cardiotonic | Digitalis |
| Tranquilizers | Rauwolfia Roots |

## Plant Sources Of Drugs In Pharmacognosy (Pharmacological Classification)

*Vinca* (Anticancer)

*Ephedra* (Antiasthmatic)

*Datura* (Antispasmodic)

*Ipecac* Roots (Antiamoebic)

*Opium* (Analgesic)

*Rauwolfia* Roots (Antiamoebic)

## An Outline Of Morphological Classification Of Crude Drugs

- The drugs **obtained from the direct parts of the plants** and **containing cellular tissues** are called as **Organized Drugs.** e. g. Rhizomes, barks, leaves etc.
- The drugs which are **prepared from plants by some intermediate physical processes** such as **incision, drying** or **extraction** with a solvent and **not containing any cellular plant tissues** are called as **Unorganized drugs.** e. g., Aloe juice, opium latex etc.

| ➢ **Sources Of Organized Drugs** | ➢ **Sources Of Unorganized Drugs** |
|---|---|
| ▪ Woods – Sandalwood | ▪ Dried latex – Opium |
| ▪ Leaves – Digitalis | ▪ Dried Juice– Aloe |
| ▪ Barks – Cinchona | ▪ Dried extracts– Agar |
| ▪ Flowering parts – Clove | ▪ Waxes – Beeswax |
| ▪ Fruits – Coriander | ▪ Gums – Acacia |
| ▪ Seeds – Nux vomica | ▪ Resins– Asafoetida |
| ▪ Roots and Rhizomes – Aconite, Turmeric | ▪ Volatile oil– Turpentine |
| ▪ Plants and Herbs – Ergot, Ephedra | ▪ Animal Products – Bees wax, Cantharides |

**Table 1: Sources Of Organized And Unorganized Drugs**

## Parts Of Plants Used In Pharmacognosy (Morphological Classification)

**Leaves of *Digitalis purpurea***

**Barks of *Cinchona***

**Flowers of Clove (*Syzygium aromaticum*)**

**Fruits of *Coriandrum sativum***

**Seeds of *Strychnos nux-vomica***

**Rhizome of Turmeric (*Curcuma longa*)**

## An Outline Of Taxonomical Classification Of Crude Drugs

Taxonomical classification is purely a botanical classification and is **based on principles of natural relationship and evolutionary developments.**

| Phyllum | Order | Family | Drugs |
|---|---|---|---|
| Thallophyta | Gelidiales | Gelidiaceae | Agar |
| Pteridophyta | Filicales | Polypodiaceae | Male fern |
| Gymnosperms | Coniferae | Pinaceae | Colophony |
| Angiosperms (Moncotyledons) | Liliflorae | Liliaceae | Colchicum |
| Angiosperms (Dicotyledons) | Umbelliflorae | Apiaceae | Coriander |

**Table 2: Taxonomical Classification of Some Crude Drugs**

- **Chemotaxonomical Classification :** The chemical examination of several plants Phytochemical evaluation have established that there is a **close link between their Chemical constituents and taxonomical status.**
  - **Solanaceae** family contains **Tropane alkaloids**
  - **Pinaceae** family contains **Oleo-resin**

# AN OUTLINE OF ALPHABETICAL CLASSIFICATION OF CRUDE DRUGS

Alphabetical classification is the simplest way of classification of any disconnected items. Crude drugs are arranged in **alphabetical order** of their **Latin and English names (common names)** or sometimes local language names (vernacular names).

Crude drugs are classified according to this system are as follows.

1. Indian Pharmacopoeia
2. British Pharmacopoeia
3. British Herbal Pharmacopoeia
4. United States Pharmacopoeia and National Formulary
5. British Pharmaceutical Codex
6. European Pharmacopoeia

## EVALUATION OF DRUGS

➢ **Evaluation of drug** means to identify and to determine its **quality** and **purity**.

- **Identity –** Identification of biological source of the drug.
- **Quality –** The quantity of the active constituents present.
- **Purity –** The extent of foreign organic material present in a crude drug.

➢ **Methods of Drug Evaluation:** The evaluation of a drug is drug done by studying its various properties.

1. **Macroscopic** Evaluation (Organoleptic evaluation)
2. **Microscopic** Evaluation
3. **Chemical** Evaluation
4. **Physical** Evaluation
5. **Biological** Evaluation

# METHODS OF EVALUATION OF DRUGS

- **Macroscopic Evaluation (Organaleptic Evaluation)** :

It is the evaluation by means of sense and includes the macroscopic appearance of the drug, its odour and taste, occasionally the sound or **'snap'** of its fracture and the '**feel**' of the drug to touch.

- **Microscopic Evaluation** :

Microscopic evaluation is not only essential in the study of adulterants in powered drugs but is also important in the **identification of the pure powdered drug**. Histological studies are made from very **thin sections**, properly mounted and sealed.

- **Chemical Evaluation** :

In this method, chemical nature of the constituents can be used as tool to device a method for the analysis of the constituents. It involves chemical tests, chemical assay and also the phytochemical investigation of the crude drugs.

- **Physical Evaluation** :

In case of crude drug extracts the application of physical constants is very rare. However, **solubility, specific gravity, optical rotation, refractive index, congealing point, melting point** and **water content** are important in the evaluation of drugs.

- **Biological Evaluation** :

It includes determination of therapeutic activity of herbal drugs by using biological models of intact animals, animal preparation, isolated living tissue or micro-organisms.

## Major pharmacological groups of plant drugs and their uses

| COMMON NAMES | SCIENTIFIC NAMES | ACTIVE AGENTS | PHARMACOLOGICAL ACTION |
|---|---|---|---|
| Red Peppers | *Capsicum annuum* | Capsaicin | Local Blood Circulation Rheumatism |
| Foxglove | *Digitalis lanata* | Digoxin | Heart Muscle Activity |
| Ginkgo | *Ginkgo biloba* | Ginkgolides | Cerebral Circulation |
| Ephedra | *Ephedra sinica* | Ephedrine | Relief Of Asthma |
| Opium Poppy | *Papaver somniferum* | Morphine | Pain Relief |
| Ergot | *Rauwolfia serpentine* | Reserpine | Hypertension |
| Quinine | *Cinchona officinalis* | Quinine | Antimalarial |
| Periwinkle | *Catharanthus roseus* | Vincristine | Hodgkin's disease |
| Pacific Yew | *Taxus brevifolia* | Taxol | Ovarian Cancers |
| Ipecac | *Cephaelis ipecacuanha* | Emetine | Amoebic Dysentery |
| Deadly Nightshade | *Atropa belladonna* | Atropine | Motion Sickness |
| Thorn Apple | *Datura stramonium* | Hyoscyamine | Epilepsy |

**Table 3: Pharmacological Uses Of Some Plant Drugs**

# Conclusion

There is an urgent need for transition from petrolium based energy system to one based on renewable resources to decrease reliance on depleting reserve of fossils fuels and to mitigate climate change. It has potential to create employment opportunities especially at all levels especially at rural level.

# Acknowledgement

I would like to express my special thanks and gratitude to my mentors, Dr. Tripti Roy and Dr. Debjani Sinha Roy, who gave me such a golden opportunity to present on this wonderful topic ‘Pharmacognosy’. Then I should mention the name of Smita Roy ma’am, from whom I first got the knowledge about this topic. Thanks to all of my classmates, who cooperated me a lot, during the presentation. And lastly, thanks to my sister, who gave me all the technical advices during the making time of the project and my mother, who supported me solely through the whole time.

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# Paper-XVII

# Phloem Loading and Unloading


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# Abstract

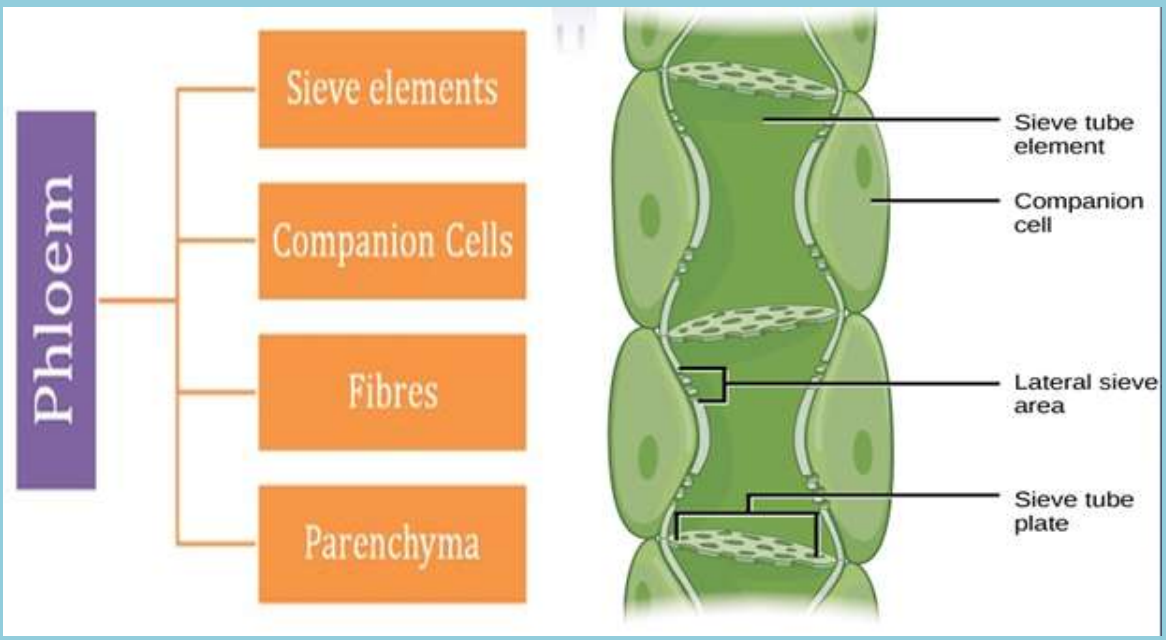
Phloem is responsible for transport of food, sugar(sucrose) from the source to the sink. This transfer of sugars (sucrose) from mesophyll cells (source) to sieve tube element in the leaf is called phloem loading. Phloem loading occur via symplastic pathway and apoplastic pathway and passive loading. On the other hand, the transfer of sugars (sucrose) from sieve tube to the sink is called Phloem unloading. Phloem unloading occur via Apoplastic, symplastic pathway. The translocation in phloem is explained
by the mass flow hypothesis. It is most accepted mechanism used for the translocation of sugars (sucrose) from source to sink.

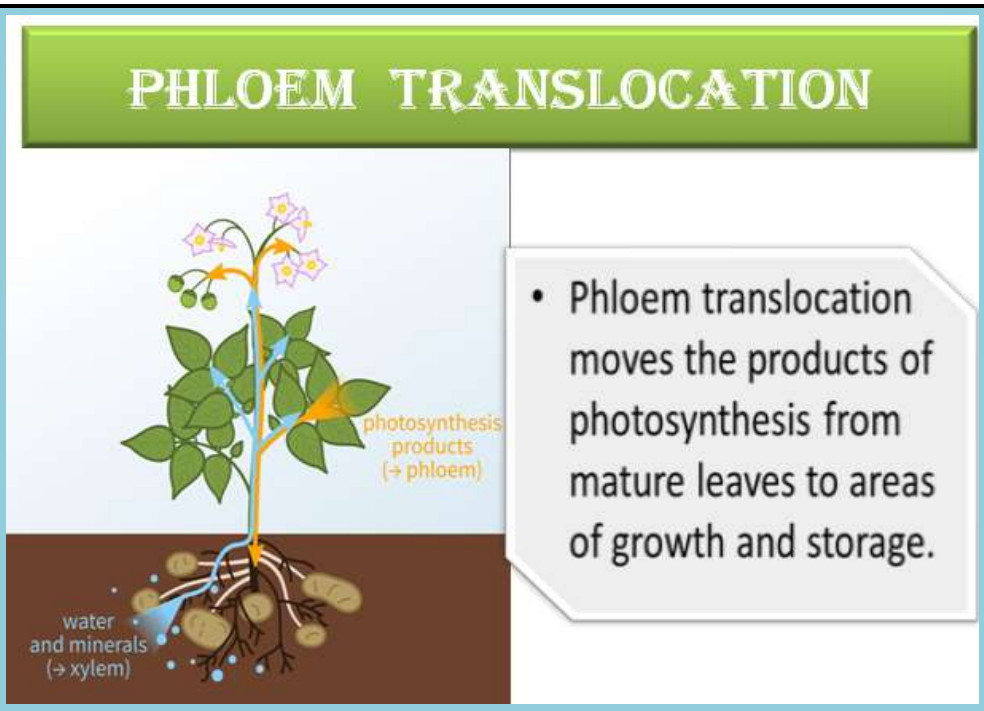
# Introduction

## What is PHLOEM ?

- The phloem is the living tissue in vascular plant.
- Phloem tissue consists of conducting cells.
- It transport the products of photosynthesis.

## Composition of phloem:






## TRABSLOCATIONS TAKES PLACE FROM SOURCE TO SINK

## Source

Sources include exporting organs, typically mature leaves that are capable of producing photosynthate.

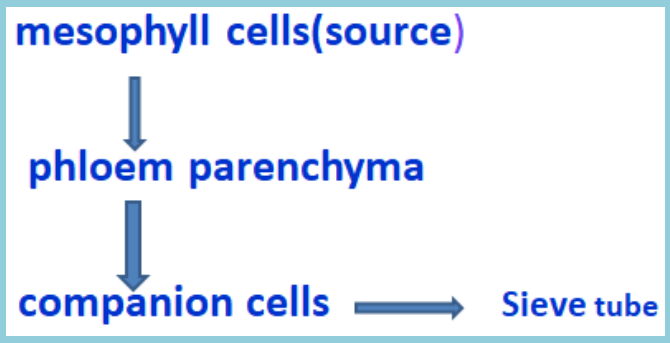
## Sink

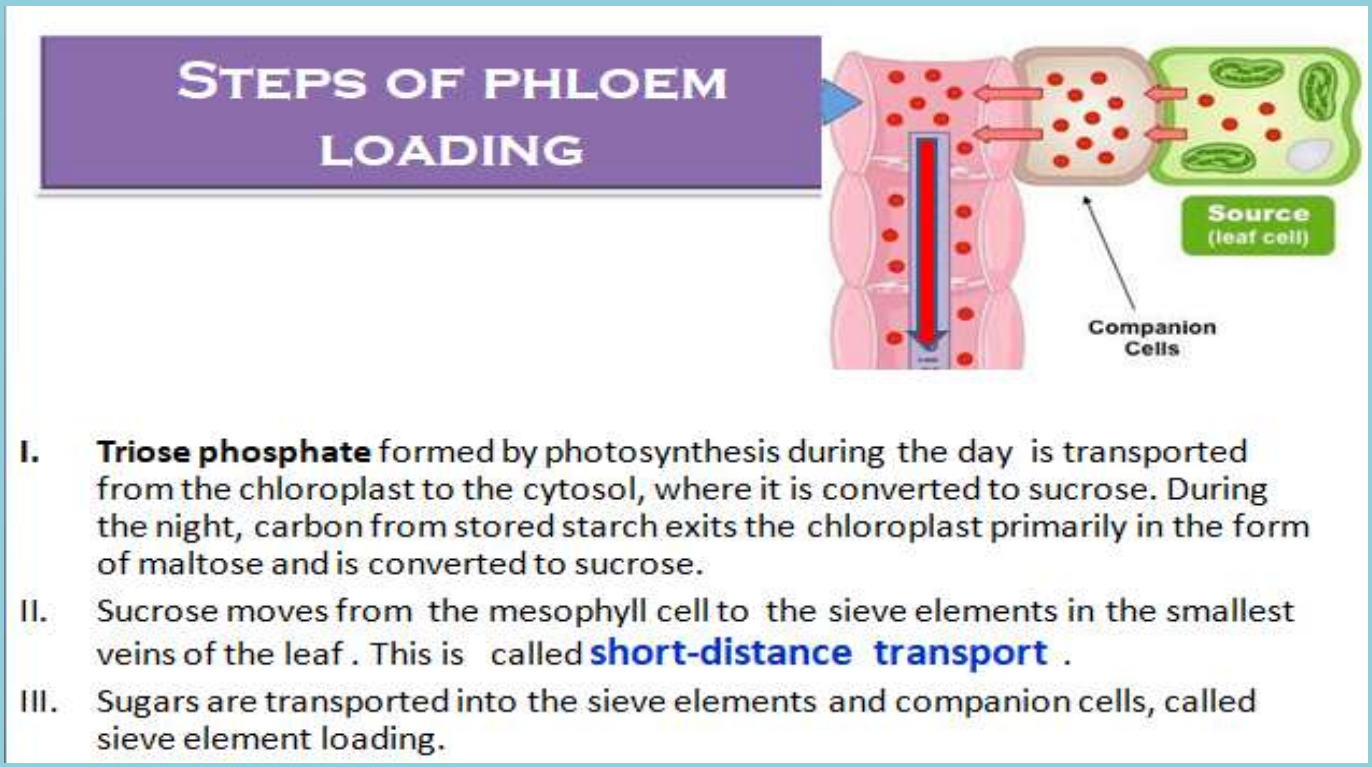
Sinks include all nonphotosynthetic organs of the plant and organs that do not produce enough photosynthetic products to support their own growth.

Roots, tubers, etc

# PHLOEM LOADING

- **The movement of photosynthate from the mesophyll cells to the sieve elements of mature leaves – is called phloem loading.**
- **Route of phloem loading:-**



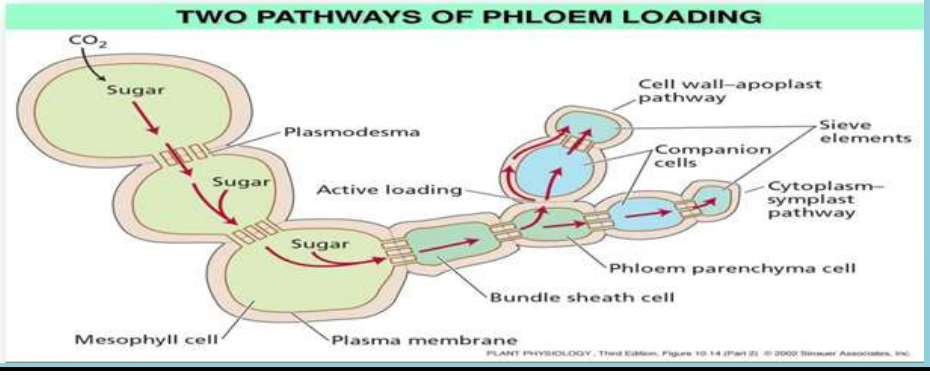




I. **Triose phosphate** formed by photosynthesis during the day is transported from the chloroplast to the cytosol, where it is converted to sucrose. During the night, carbon from stored starch exits the chloroplast primarily in the form of maltose and is converted to sucrose.

II. Sucrose moves from the mesophyll cell to the sieve elements in the smallest veins of the leaf . This is called **short-distance transport** .

III. Sugars are transported into the sieve elements and companion cells, called sieve element loading.

- **Phloem loading can occur via the apoplast or symplast**
- **Apoplast loading :-** loading through **Cell wall**
- **Symplast loading:-** loading through **plasmodesmata**



## Phloem loading in the apoplastic pathway involves a sucrose–H+ symporter

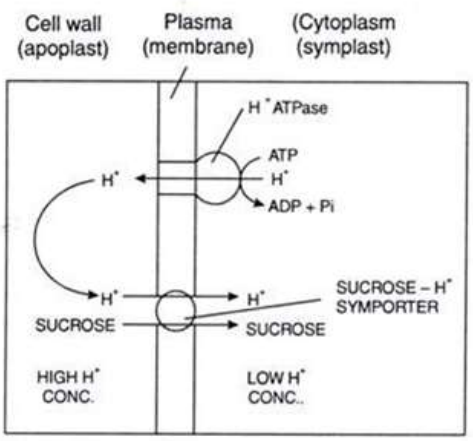



Fig. 15.5. Sucrose –H+ symport or cotransport mechanism.

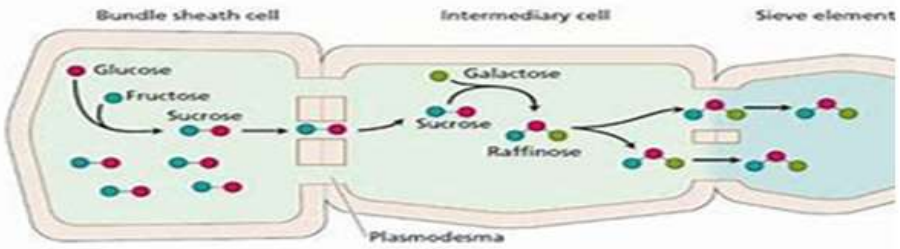
- Example: **Mainly herbaceous**

1. In the model of sucrose loading into the symplast of the sieve element– companion cell complex, the plasma membrane ATPase pumps protons out of the cell into the apoplast, establishing a higher proton concentration in the apoplast.
2. The energy in the proton gradient is then used to drive the transport of sucrose into the symplast of the sieve element–companion cell complex through a sucrose–H+ symporter.

- Plants that load sugars apoplastically into the phloem may also load amino acids and sugar alcohols (sorbitol and mannitol) actively.

## The polymer-trapping model explains symplastic loading in plants

1. **Sucrose synthesized in the mesophyll, diffuses from the bundle sheath cells into the intermediary cells through the plasmodesmata,**
2. **In intermediary cells, raffinose is synthesized from sucrose .**
3. **Due to large size, raffinose can not diffuse back into the mesophyll but they can diffuse into the sieve element.**
4. **As a result, the concentration of transport sugar rises in the intermediary cells and the sieve elements.**



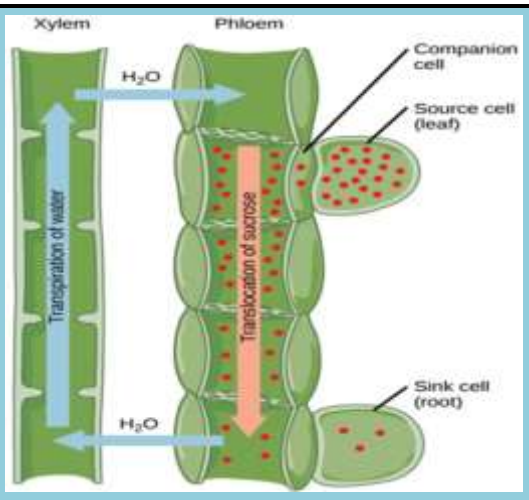

Example: **Herbs and woody species**

## Passive loading

- Passive symplastic phloem loading has recently been recognized as a mechanism that is widespread among plant species.
- Several tree species possess abundant plasmodesmata between the sieve element– companion cell complex and surrounding cells but do not have intermediary-type companion cells and do not transport raffinose and stachyose.

Example : **Apple (Malus domestica) trees are among the species that fall into this category.**

**NOTE:** The sieve elements and companion cells are considered a functional unit, called **the sieve element–companion cell** complex.



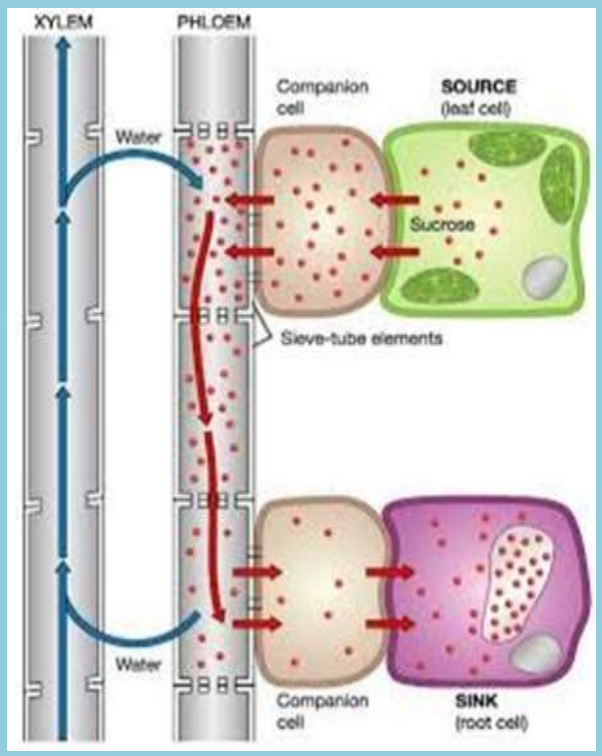

# MECHANISM OF TRANSLOCATION IN PHLOEM

•The most widely accepted mechanism of phloem translocation in angiosperms is the pressure-flow model.

•The pressure-flow model, first proposed by Ernst Munch in 1930.

• He states that a flow of solution in the sieve elements is driven by an osmotically generated pressure gradient between source and sink .Phloem loading at the source and phloem unloading at the sink establish the pressure gradient.

•The pressure-flow model is an example of a passive mechanism.



# THE PRESSURE FLOW MODEL

•In source tissues, an accumulation of sugars in the sieve elements generates a low (negative) solute potential and causes a huge drop in the water potential . In response to water potential gradient, water enters the sieve elements and causes the turgor pressure to increase.

•At the receiving end of the translocation pathway, phloem unloading leads to a lower sugar concentration in the sieve elements, generating a higher (less negative) solute potential in the sieve elements of sink tissues.

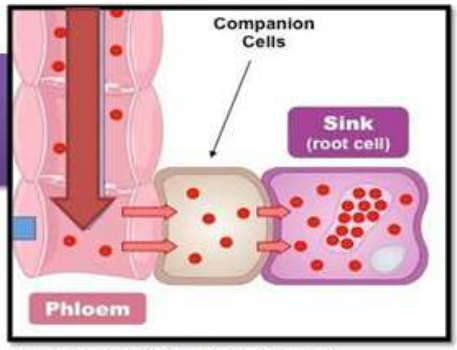
•As the water potential of the phloem rises above that of the xylem, water tends to leave the phloem in response to the water potential gradient, causing a decrease in turgor pressure in the sieve elements of the sink.

# PHLOEM UNLOADING

❑ **Sieve tube ⟶ Sink**

- **The movement of sucrose from sieve-tube into the sink is called -phloem unloading.**

## STEPS OF PHLOEM UNLOADING




- 1. Phloem unloading: This is the process by which imported sugars leave the sieve elements of sink tissues.
- 2. After unloading, the sugars are transported to cells in the sink by means of a **short-distance transport pathway**. This pathway has also been called post–sieve element transport.
- 3. The final step, sugars are stored or metabolized in sink cells.

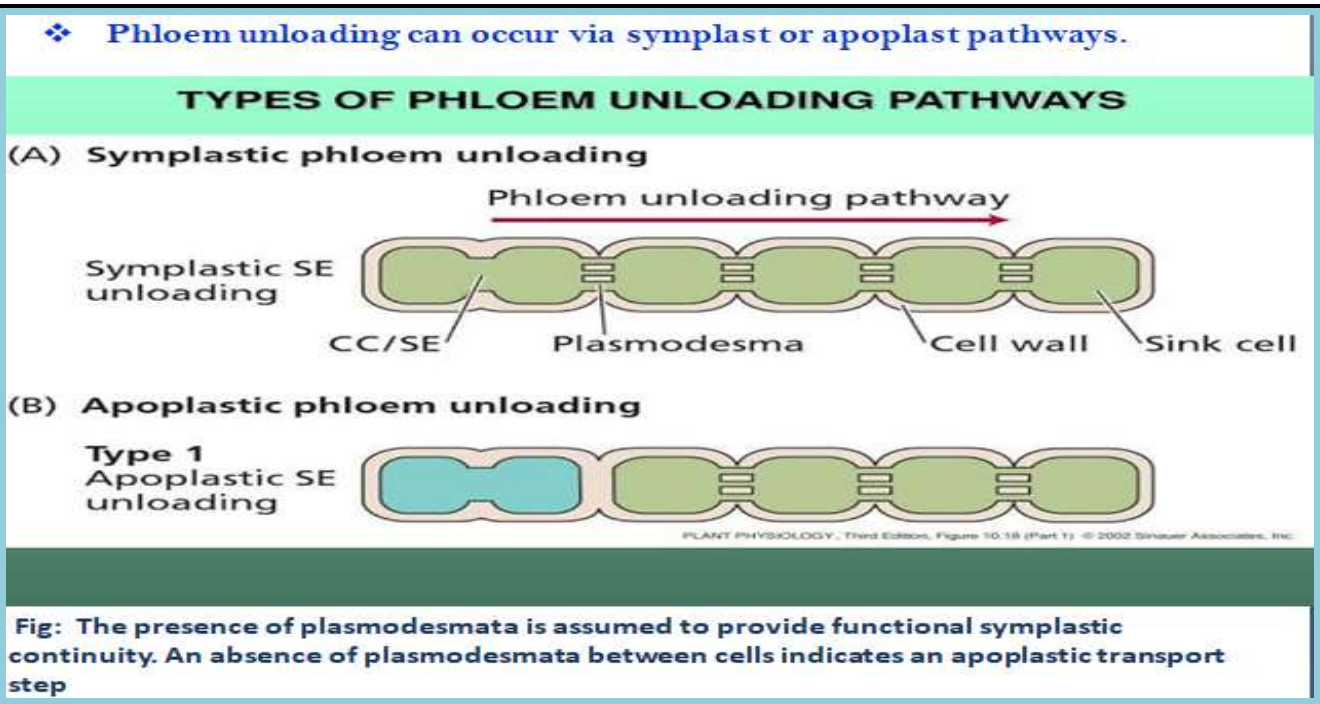



Fig: The presence of plasmodesmata is assumed to provide functional symplastic continuity. An absence of plasmodesmata between cells indicates an apoplastic transport step

# Conclusion

Phloem loading and unloading is very important phenomenon for the movement of sugar from source to sink. In angiosperms, the Pressure flow model is widely accepted phloem transport mechanism.

# Acknowledgement

I would like to express my gratitude to Dr.Tripti Roy, Dept. of Botany Bethune college, Kolkata for giving me the opportunity to present this topic. It has been a learning experience for me. Thankyou ma'am for your support.

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# Paper-XVIII

# Mode Of Action Of Auxin


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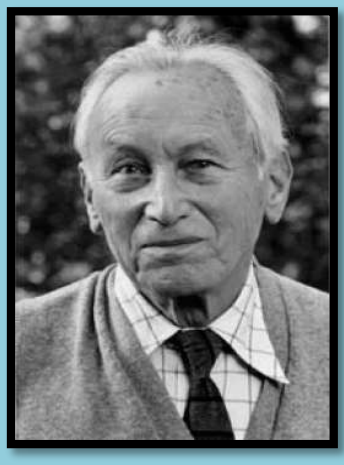
# Abstract

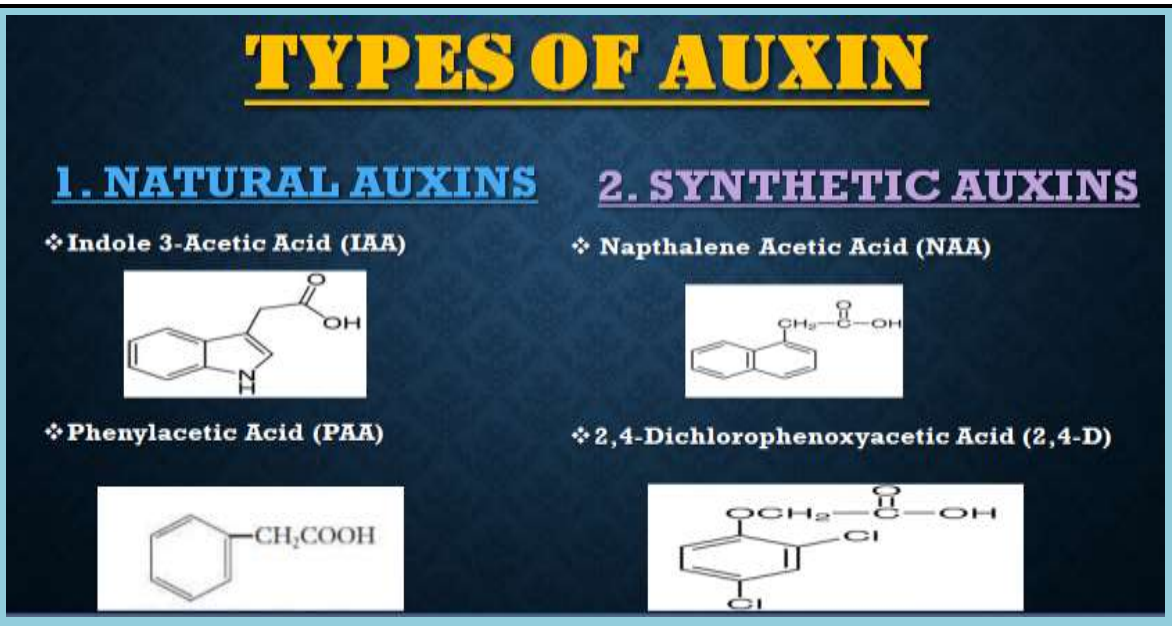
Auxin stimulates cell elongation by stimulating wall-loosening factors, such as expansin, to loosen cell walls. $ABP_1$ (Auxin Binding Protein 1)-Docking protein complex then activates the ATPase-proton pump. $ABP_{57}$ activates the pre-existing plasma membrane $H^+$-ATPases. New $H^+$-ATPases also synthesized on the plasma membrane. Auxin also induced gene de-repression.

# Introduction

Auxin comes from the Greek word “auxein” which means “to increase”. The term auxin was first used by Frits Went in 1926.

Kenneth V. Thimann (1948) defined auxin as “an organic substance which promotes growth along the longitudinal axis when applied in low concentrations in shoots of plants freed as far as possible from their inherent growth-promoting substances”.






# GENERAL MODE OF ACTION OF AUXIN

- Auxin binds to a highly specific receptor which are proteinaceous , bound to plasma membrane or cytoplasm.
- The binding of auxin to receptor brings about a change in the receptor conformation , aggregation and/or arrangement.
- The change in receptor conformation initiates a signal transduction pathway mediated by secondary messengers.
- This signal transduction pathway:

i. Activates/deactivates enzymes,protein kinases and/or
ii. Up-regulates protein/enzyme synthesis and/or
iii. Activates synthesis of novel mRNA and/or
iv. Activates specific genes , specific for auxin action.

- Auxin induces rapid responses like cell elongation mediated by plasma membrane bound $H^+$-ATPase activity.

❑ Auxin increases rate of proton extrusion by three possible mechanisms:-

I. Activation of pre-existing plasma membrane bound $H^+$-ATPases.
II. Synthesis of new plasma membrane bound $H^+$-ATPases.
III. Increase the amount of $H^+$-ATPases in the plasma membrane.

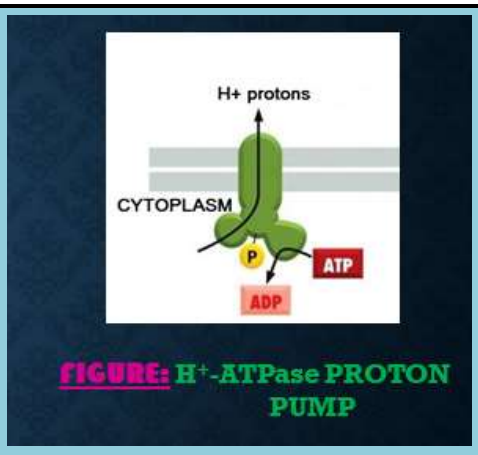



FIGURE: H⁺-ATPase PROTON PUMP

# ACID-GROWTH HYPOTHESIS

## HISTORY

- In 1970, R.Cleland and D.Rayle proposed a theory to explain increases in cell wall extensibility stimulated by auxin and suggested that auxin causes acidification of cell wall by stimulating proton excretion from cells.
- At the same time, A.Hager, working in Germany prposed a same theory but suggested that auxin stimulated proton excretion by activating a plasma membrane bound ATPase proton pump.
- The combined Cleland-Hager proposals are known as the "**ACID-GROWTH HYPOTHESIS**".

## MECHANISM

1. Auxin activates ATP-proton pumps located in the plasma membrane.
2. Expansin, a pH-dependent wall-loosening protein made cell walls more extensible and thereby the plant cells enlarge during acid growth.
3. Cellulose microfibrils loosens.
4. Cell volume increases by turgor and osmosis.
5. Involvement of auxin causes protons to be pumped out into the cell wall.
6. The cell wall solution becomes more acidic.
7. Acidification causes increment in expansin activity which allows turgor-induced cell expansion.
8. The cell wall become more extensible and undergo wall stress relaxation.
9. As a result,the cell becomes able to take up water and to expand.

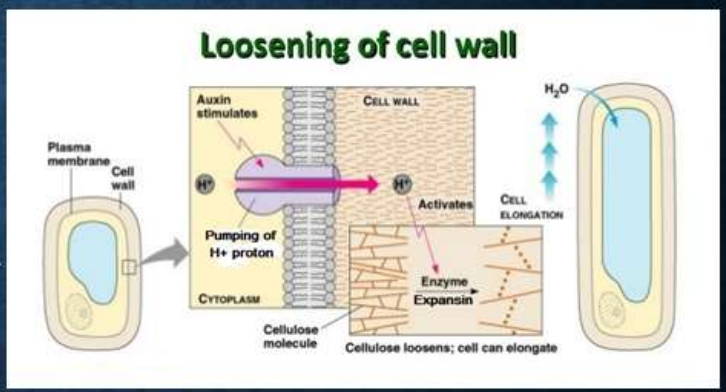



FIGURE: Role Of Auxin In Acid-Growth Hypothesis For Cell Enlargement

## ❑Auxin itself does not bind to the ATPase.

**THEN HOW IT WORKS???** 

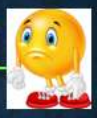
### AUXIN-BINDING PROTEIN 1 (ABP1)

a. It is an auxin receptor, isolated from maize (*Zea mays*).

b. It is a membrane-associated protein.

c. ABP1 is a 43kDa glycoprotein dimer of 22 kDa subunits.

d. It has been localized primarily in the endoplasmic reticulum,but small populations are also found associated with the plasma membrane and in the cell wall.

### PROBLEMS OF ABP1 

I. It is predominantly found in the lumen of endoplasmic reticulum and very small population on the plasma membrane.

II. It has no lipophilic membrane-spanning domain.

**THEN HOW IT WILL ANCHORS ???** 

- ABP1 forms a complex with a transmembrane docking protein.
- The docking protein provides the necessary solubility to anchor ABP1 to the membrane.
- The ABP1-docking protein complex is then exported to the plasma membrane.

### ACTIVATION OF ATPase-PROTON PUMP

- The ABP1-docking protein complex is itself inactive.
- Attachment of an auxin molecule activates the protein.
- Auxin also activates the enzyme phospholipase $A_2$ ($PLA_2$).
- The products of $PLA_2$ i.e., Lysophospholipids (LPC) and Fatty acids (FA).
- LPC activates Protein Kinase (PK).
- PK activates a proton-ATPase by phosphorylation dependent mechanism.

**THE DOCKING PROTEIN MIGHT BE A GCPR RECEPTOR IN THE FAMILY OF G-PROTEINS.**

*Vanadate*, a proton kinase inhibitor.

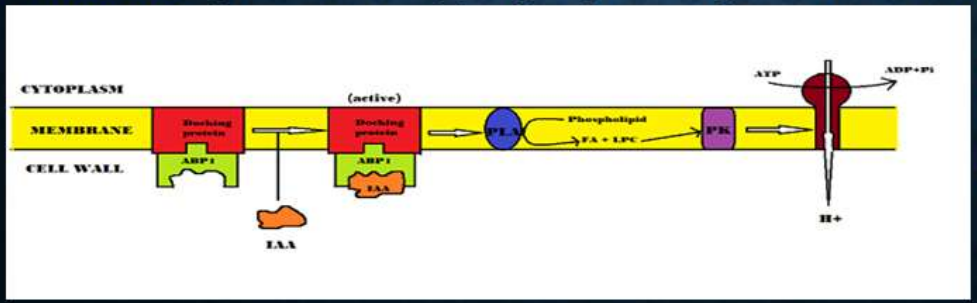



**FIGURE:** A HYPOTHETICAL SIGNAL TRANSDUCTION CHAIN LINKING AUXIN WITH ACTIVATION OF THE ATPase-PROTON PUMP.

## ACTIVATION OF PRE-EXISTING PLASMA MEMBRANE H⁺-ATPASES

- $ABP_{57}$, an ABP isolated from rice binds directly to plasma membrane H⁺-ATPase.
- It stimulates proton extrusion only in presence of auxin.
- In absence of auxin, the C-terminal inhibitory domain repressed the activity of H⁺-ATPase by blocking the catalytic site.
- Auxin when binds to $ABP_{57}$ causes a conformational change in $ABP_{57}$ and then interacts with the inhibitory domain and activates the H⁺-ATPase enzyme.
- Auxin when binds to second site, it decreases interaction with inhibitory domain and inhibits the H⁺-ATPase enzyme.

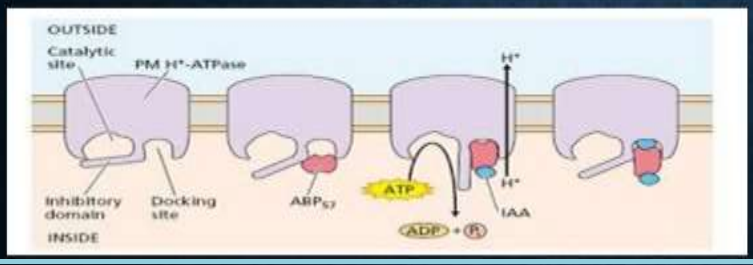



**FIGURE:** MODEL FOR THE ACTIVATION OF PLASMA MEMBRANE H⁺-ATPASE BY $ABP_{57}$ AND AUXIN.

## SYNTHESIS OF NEW H⁺-ATPASES ON THE PLASMA MEMBRANE

- Increase in the amount of plasma membrane H⁺-ATPases in corn coleoptiles was detected only after 5 minutes of auxin treatment, and a doubling of the H⁺-ATPase was observed after 40 minutes of treatment.
- A stimulation by auxin of an mRNA for the H⁺-ATPase was demonstrated in the non vascular tissues of the coleoptiles.

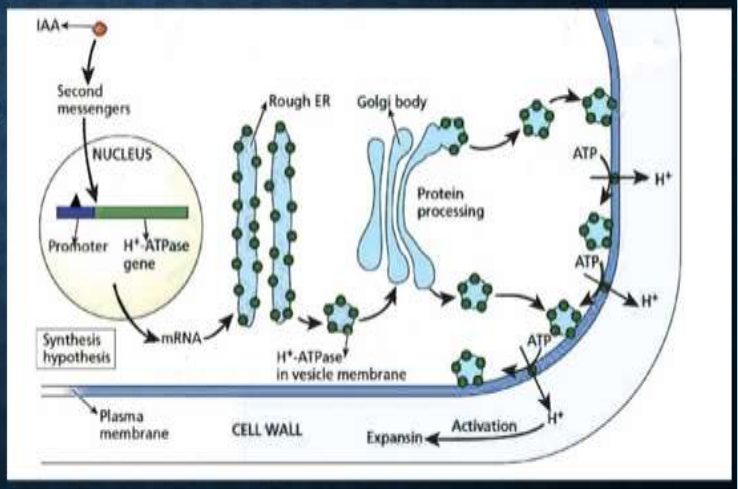



**FIGURE:** MODEL FOR SYNTHESIS OF NEW H⁺-ATPases ON THE PLASMA MEMBRANE.

## AUXIN-INDUCED GENE DE-REPRESSION

## FIVE MAJOR CLASSES OF AUXIN-RESPONSIVE GENES

### A) GENES INVOLVED IN AUXIN-REGULATED GROWTH AND DEVELOPMENT:

#### 1. THE *AUX/IAA* GENE FAMILY:-

- Auxin stimulates the action of *AUX/IAA* genes after 5 to 60 minutes of treatment.
- This gene family encodes transcription factors that function as repressors or activators of the expression of auxin-inducible genes.

#### 2. THE *SAUR* GENE FAMILY:-

- Auxin stimulates the action of *SAUR* genes after 2 to 5 minutes of treatment.
- These genes are found in soybean.
- This gene family helps in the lateral transport of auxin.
- Cycloheximide inhibits this gene family.

#### 3. THE $GH_3$ GENE FAMILY:-

- Auxin stimulates the action of $GH_3$ genes within 5 minutes of treatment.
- These genes are found in soybean and *Arabidopsis*.
- Mutation in *Arabidopsis* $GH_3$-like genes resulted in dwarfism.

# B) STRESS RESPONSE GENES:

## 1. GENES ENCODING GLUTATHIONE S-TRANSFERASES (GSTs):-

- Glutathione S-transferase is a class of proteins stimulated by various stress condition.
- These genes are induced by elevated auxin concentrations.

## 2. GENES ENCODING 1-aminocyclopropane-1-carboxylic acid (ACC) SYNTHASE:-

- This is the key enzyme in the ethylene biosynthetic pathway.

# MECHANISM OF GENE DE-REPRESSION

### ❖ IN PRESENCE OF HIGH LEVEL OF AUXIN

i. Binding of auxin response factor (ARF) to the DNA in the promoter region of auxin-responsive genes.
ii. Prevention of gene transcription by *AUX/IAA* repressor protein.
iii. Formation of auxin-TIR1 complex.
iv. Dissociation of *AUX/IAA* from the ARF by TIR1.
v. De-repression of gene by removal of *AUX/IAA* from ARF.
vi. Gene transcription occurs.
vii. Recruitment of *AUX/IAA* to the E3 ubiquitine-ligating enzyme by TIR1.
viii. Polyubiquination of *AUX/IAA*.
ix. Recruitment of ubiquinated *AUX/IAA* proteins to the 26S proteasome.
x. Degradation of *AUX/IAA* proteins.

### ❖ IN PRESENCE OF LOW LEVEL OF AUXIN

i. TIR1 unable to bind with the repressor.
ii. Accumulation of repressor proteins.
iii. Gene transcription does not occur.

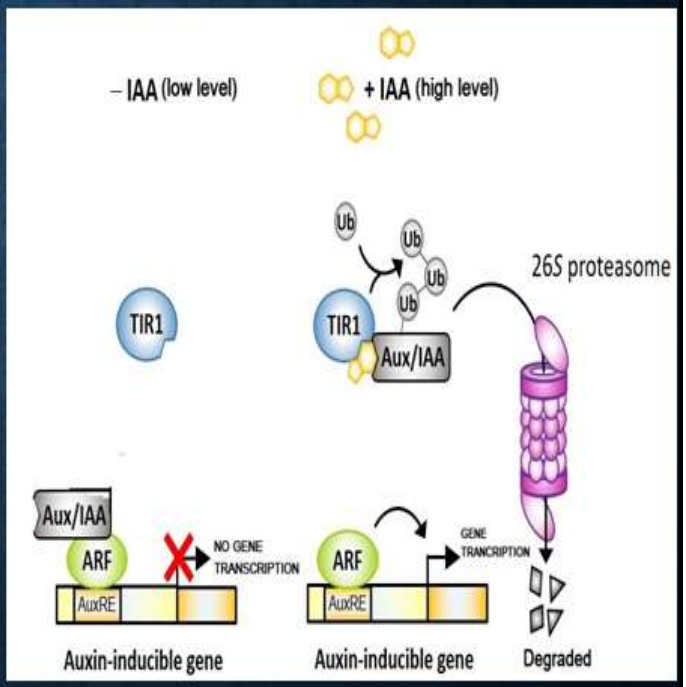



**FIGURE: AUXIN INDUCED GENE DE-REPRESSION**

# Conclusion

The plant hormone auxin triggers cell growth and elongation of the plant In the elongation process, auxin alters the plant wall plasticity making it easier for the plant to grow upwards. Auxin also influences gene de-repression.

# Acknowledgement

I would like to express my special thanks and gratitude towards Dr. Tripti Roy, Associate Professor of the Department of Botany, Bethune College, Kolkata, and Dr. Debjani Sinha Ray, Associate Professor of the Department of Botany, Bethune College, Kolkata, for giving me the golden opportunity to do this wonderful work and for expressing myself through my topic. For this work, I came to know about so many new things.

Thank you Ma'am for your support and guidance and thank you my fellow classmates for your support and encouragement.

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# Paper-XIX

# Energy
# With Special Emphasis On
# Renewable Energy


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# Abstract

A renewable energy source means energy that is sustainable - something that can't run out, or is endless, like the sun. When you hear the term 'alternative energy' it's usually referring to renewable energy sources too. It means sources of energy that are alternative to the most commonly used non-sustainable sources - like coal.

The most popular renewable energy sources currently are:

1. Solar energy
2. Wind energy
3. Hydro energy
4. Tidal energy
5. Geothermal energy
6. Biomass energy

# Introduction

Considering that the major component of green house gases (GHGs) is carbon dioxide, there is a global concern about reducing carbon emissions. In this regard different policies could be applied to reduce carbon emissions, such as enhancing RENEWABLE ENERGY deployment and encouraging technological innovations. In addition supporting mechanisms such as feed in tariffs ,renewable portfolio standards and tax policies , are employed by government to develop RENEWABLE ENERGY generation along with implementation energy use efficiency for saving energy.

# WHAT IS ENERGY :

Energy, is the capacity to do work and is required for life processes. An energy resource is something that can produce heat, power life, move objects or produce electricity. Matter that stores energy is called as fuel. The whole development of civilization is based on the availability of energy. Energy is present in different forms and it has been further modified from time to time to suit the requirements of mankind.

## ENERGY AS A NATURAL RESOURCES :

The term natural resource refers to any quality or substance that is valued by humans, exist without any actions of humankind. This include the sources of valued characteristics such as commercial and industrial use, aesthetic value, scientific interest, cultural value.

Energy is all around us and manifest itself in many different forms – heat; light; sound; magnetism; gravity; movement and all life functions. It is everywhere in great abundance. Since the beginning of time, nature has been producing and reproducing energy in quantities, that we could never begin to use it all, not even with the most advanced technology.

## ENERGY IS TWO TYPES

1. RENEWABLE ENERGY.
2. NON RENEWABLE ENERGY

# RENEWABLE ENERGY

A **renewable energy** source means energy that **is sustainable** something that **can not run out**, or is **end-less**, which are naturally replenished on a human time-scale, including carbon natural sources like **the sunlight, wind, rain, tides, waves & geothermal heat.** It means sources of energy that are alternative to the most commonly used non-sustainable sources-like coal.


Solar energy


wind energy


Biomass energy


Tidal energy


Hydro energy


Geothermal energy

# WHAT IS SOLAR ENERGY ?

Solar power energy from the sun that is converted into thermal or electrical energy. This energy is in the form of solar radiation which makes the production of solar electricity possible.
Solar energy is the cleanest and most abundant **renewable energy** source available, and the U.S. has some of the richest solar resources in the world.Solar technologies can harness this energy for a variety of uses, including generating electricity, providing light or a comfortable interior environment, and heating water for domestic, commercial or industrial use.

# HOW DOES SOLAR WORK ?

The amount of sunlight that strikes the easth's surface in an hour and half is enough to handle the entire world's energy consumption for a full year. Solar technologies convert sunlight into electrical energy either through **photovoltaic (PV) panels** or through mirrors that concentrate solar radiation. The most common solar cells are made from silicon(semiconductor)., which is sandwiched between conductive layers. A silicon solar cell uses two different layers of silicon- an n-type silicon has extra electrons and p-type silicon has extra spaces for electrons, when both types of silicon meet electron can wander across the p/n junction. This energy can be used to generate or be stored in batteries or thermal storage.


Photovoltaic panels

# USES OF SOLAR ENERGY :

- As heat for making hot water, heating buildings and cooking.
- To generate electricity with solar cells or heat engines.
- Solar space heating systems include powering radiant floors or pairing with a **Forced Hot Air system (F.H.A)** to heat a home.
- **Solar-powered vehicles** may be the future,with existing application including buses,trains,airplanes,and **race cars** build by students in **Australia**, **U.S** A fully solar powered car is even slated for commercial **release in 2019**.
- Solar ventilation solutions such as **solar attic fans** can reduce the burden of HVAC by helping to cool home during summer.


SOLAR SPACE HEATING


SOLAR VENTILATION


SOLAR CAR

# WHAT IS WIND ENERGY ?

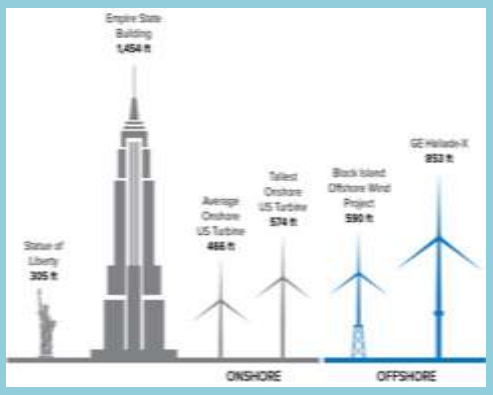
The wind is a free clean and readily available renewable source of energy.Ech day around the world, wind turbines are capturing the wind's power and converting it to electricity.Wind powervgeneration plays an increasingly important role in the way we power our eorld-in a clean sustaionable manner.

# HOW WIND ENERGY IS CREATED ?

Wind turbines allow us to harness the power of the wind and turn into energy. When the wind blows, the turbine's blades spin clockwise, capturing energy. This triggers the main shaft of the wind turbine, connected to a gearbox sends the wind energy to the generator, converting it to electricity. Electricity than travels to a transformer, where voltage levels are adjusted to match with the grid.

WIND ENERGY


**COMPONENTS OF WIND TURBINE**




**HEIGHT OF THE TOWER**

**BLADE TRANSPORATION**

# USES OF WIND ENERGY:

- Wind turbines are installed to capture the power of the wind and be able to convert it to energy. The wind turbines found on wind farms, such as individual wind turbines people use to generate power for their home.
- The wind energy is used to propel the sail boats in river and seas to transport people and materials from one place to another.
- Wind energy is used to run pumps to draw water from the grounds through wind mills.
- Wind energy has also been used to run flourmills to grind the grains like wheat and corn into flour.

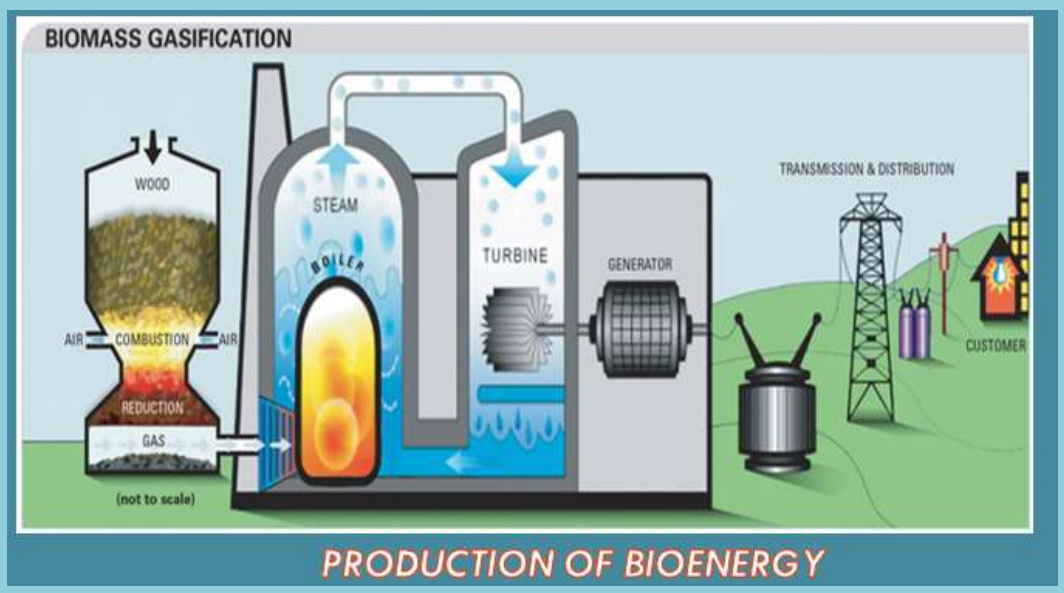
# WHAT IS BIOMASS ENERGY ?

Biomass refers to the mass of living organisms, including plants, animals and microorganisms. Biomass energy is energy generated or producted by living or once-living organisms. biomass contains stored chemical energy from the sun. Plants produce biomass through photosynthesis. Biomass can be burned directly for heat or converted to renewable liquid and gaseous fuels through various processes.

# HOW BIOMASS ENERGY IS CREATED ?

Biomass contain energy first derived from the sun. Plants absorb the sun's energy through photosynthesis, and convert carbon dioxide and water into nutrients (carbohydrates). Biomass can be burned to create heat (direct), converted into electricity (direct), or processed into bio-fuel(indirect)

BIOMASS ENERGY




# USES OF BIOMASS ENERGY :

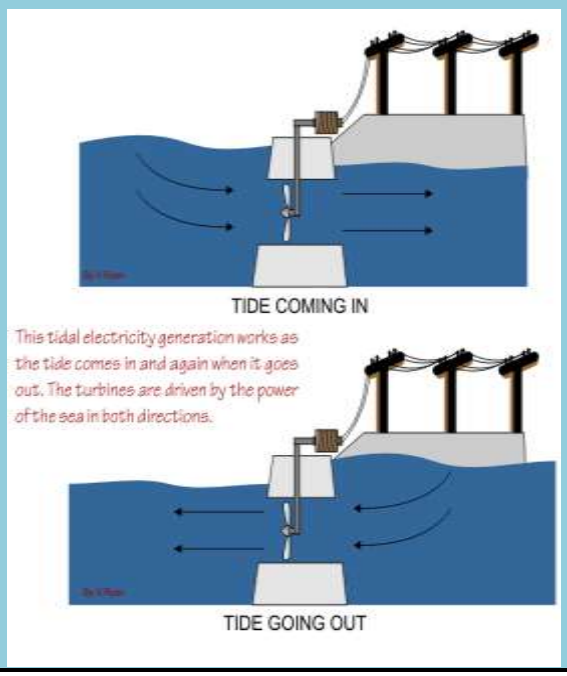
- Used in the production of Cellulosic Ethanol.
- Used in the production of Bio-diesel.
- Gasification process to produce synthesis gas (syngas).

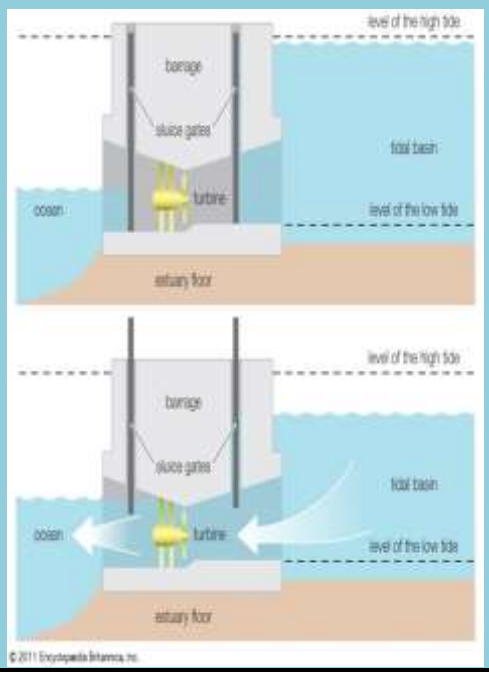

# WHAT IS TIDAL ENERGY?

**Tidal energy** is a form of hydro **power** which converts the **energy** obtained from tides into other useful energies (electricity).The **tidal energy** is the result of the sun and moon's influence over the ocean. **Tidal power** utilizes energy contained in tides to produce electricity.

# HOW TIDAL ENERGY IS CREATED?

**Tidal** barrages look like traditional hydropower dams. Turbines located along the buttom of the barrage are turned with the incoming and and outgoing tides. During an incoming high tideswater flows over the turbines. As the water rises.then, the water flows back through the turbines as it become low tide.



# USES OF TIDAL ENERGY :

- Tidal electricity - like other forms of energy , use in the generation of Electricity.
- The main usage of tidal energy is in the generation of electricity.
- The movements of turbines due to tidal energy was used in the crush grains.
- Tidal barrages can prevent damage to the coast during high storms and also provide an easy transport method between two arms of bay or an estuary on which it is built.

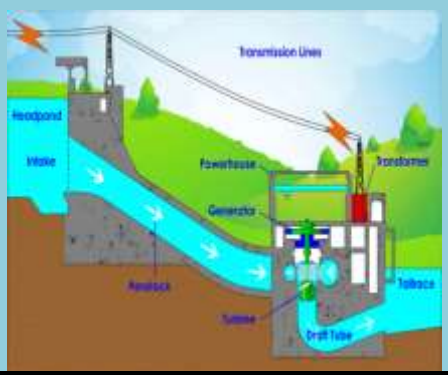
# WHAT IS HYDRO ENERGY ?

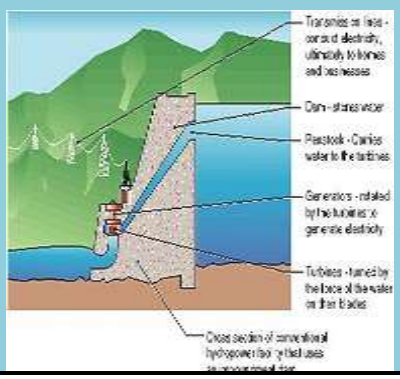
Hydroelectricity is a form of energy that harnesses the power of water in motion-such as water flowing over a waterfall-to generate electricity.

# HOW HYDRO ENERGY IS CREATED?

The main ingredients of hydroelectric power plants are dams to create reservoirs where the water is stored. This water is then released through turbines and spun to activate gnerators and create electricity.

**HYDRO ENERGY**



# USES OF HYDRO ENERGY:

- Generating clean electricity is the primary use of hydropower energy. Hydropower dams divert water for irrigation in Agriculture.
- Hydropower energy is also employed in flood risk management.
- SAWMILL & GRISTMILL.

SAWMILL

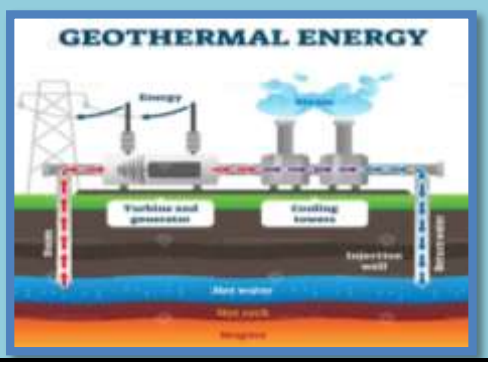
GRIST MILLS

# WHAT IS GEOTHERMAL ENERGY?

**Geothermal energy** is heat within the earth. The word geothermal comes from Greek word **geo** (earth) and **therme** (heat).**Geothermal energy** is the heat produced deep in the earth's core.It is containedin the rocks and fluids beneath the earth's crust and can be found as far down to the earth's hot molten rock , magma.

# HOW GEOTHERMAL ENERGY IS CREATED?

**Geothermal power** plants use to steam to **produce electricity**. The steam comes from reservoirs of hot water found a few miles or more below the earth's surface. The steam rotates a turbine that activates a generator, which produces **electricity**.



# USES OF GEOTHERMAL ENERGY:

- Direct use of hot water from geothermal resources can be used to provide heat for industrial processes, crop drying.
- Space heatings for various kinds of buildings.
- Generation of electric power.
- Balneological use.(use of hot water in thermal baths)

# Conclusion

There is an urgent need for transition from petrolium based energy system to one based on renewable resources to decrease reliance on depleting reserve of fossils fuels and to mitigate climate change. It has potential to create employment opportunities especially at all levels especially at rural level.

# Acknowledgement

I would like to express my special thanks and gratitude to my mentors, *Dr.Tripti Roy* and *Dr. Debjani Sinha Roy*, who have given me such a golden opportunity to do the presentation on this wonderful topic *"RENEWABLE ENERGY"*. Thanks to all of my classmates, who cooperated me a lot, during this presentation.

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# Paper-XX

# STOMATAL OPENING AND CLOSING MECHANISM

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# Abstract

Most of the transpiration occurs through the stomatal movement. Stomata that are present in the leaf surface of plants consist of pore, guard cells, subsidiary cells and epidermal cells. The excess water of plant can exit through the stomatal pore. The guard cells are continuously swelling and contracting that results in the changes of pore dimensions. Two main theories are there to describe how the stomata close and open- 1) starch sugar interconversion theory , 2) proton-potassium exchange pump theory. In addition to this, there are also some influencers which have immense effect on stomatal opening and closing. They are blue light, zeaxanthin, carbon dioxide and Abscisic acid. Here are some details about these topics in these slides.

# Introduction

Stomata (singular- stoma) consist of minute pores that allows communication between leaf and environment and a pair of kidney shaped guard cells that surrounds the pore. The guard cells can be flanked by specialized subsidiary cells that separate them from epidermal cells. Only the guard cells contain chloroplast among them.

Guard cells are continuously swelling and contracting that results the changes in pore dimensions. Distribution of stomata in plants are not uniform. In dicot they present mainly in lower surface, in monocot stomata are distributed in both surfaces. Floating leaves have stomata in their upper surfaces only.

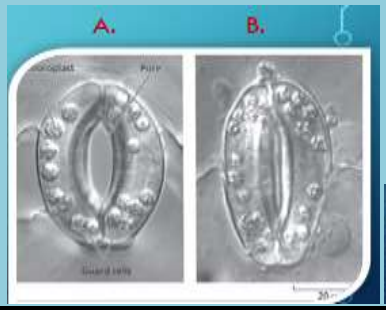



Figure 1: A. Stomata open in presence of light observed in *Vicia faba*. B. Closed state of stomata.

# STARCH – SUGAR INTERCONVERSION THEORY

•According to this classiical theory, proposed by Sayre(1923) , during day time guard cells contain starch in much amount than that of night.

• The starch is hydrolyzed to form Glucose - 1- phosphate under high pH caused due to reduced carbon dioxide concentration.

•The reduction of $CO_2$ in day time occurs due to photosynthesis and as a product of photosynthesis sugar concentration increases and the osmotic pressure as well.

• As a result guard cells become hypertonic to epidermal cells and water enters into the guard cells and makes them turgid and stomata open.

• During night the reverse process occurs as there is no photosynthesis. Guard cells becomes hypotonic so that it releases water and becomes flaccid, then the stomata close.

• But according to Steward (1964) , the conversion of starch to glucose-1 –phosphate is not sufficient. It should be converted to glucose in order to increase sufficient osmotic pressure.

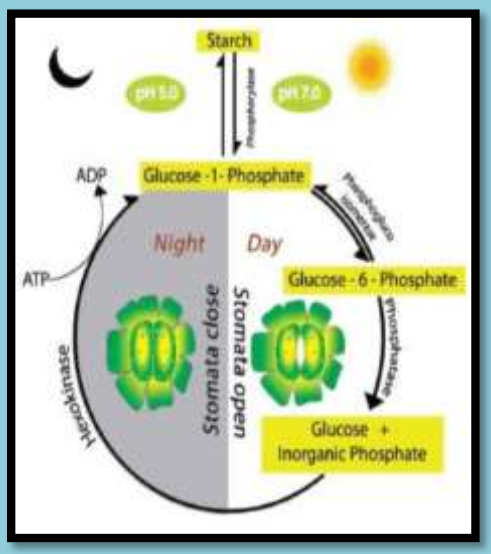



**Figure 3 : Diagrammatic representation of how starch and sugar are inter=converted and the stomata open.**

# PROTON-POTASSIUM EXCHANGE PUMP MECHANISM

According to this mechanism, proposed by Levit(1974), $K^+$ ions are transported into the guard cells in the presence of light. The sequence of events taking place are as follows:

- The $H^{+,}$ formed by dissociation of malic acid are pumped out from the guard cells to the epidermal cells.
- To counter the exit of protons, $K^+$ ions enters the guard cells from the surrounding mesophyll cells.
- $K^+$ ions react with the malate ions present in the guard cells to form potassium malate.
- Potassium-malate causes increase in the osmotic pressure of guard cells causing entry of water into the guard cells as a result of which the stoma opens.
- At night the dissociation of potassium - malate takes place and $K^+$ ions exit out of guard cells causing loss of water, so the stoma closes.

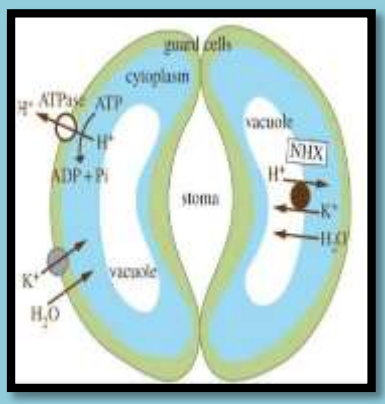



**Figure 4 : Proton-potassium fluxes that regulates stomatal movement.**

# BLUE LIGHT STIMULATED STOMATAL MOVEMENT

- The stomatal response to blue light is rapid and reversible and localized only in the guard cells.

• Blue light stimulates an $H^+$ - ATPase at the guard cell plasma membrane, generating an electro-chemical gradient that drives ion uptake.

• This ions in guard cell protoplast provides a mechanical force working against the rigid wall that distorts the guard cells and drives increasing stomatal aperture.

• Blue light modulates guard cell osmoregulation by means of its activation of proton pumping , solute uptake and stimulation of the synthesis of organic solutes.

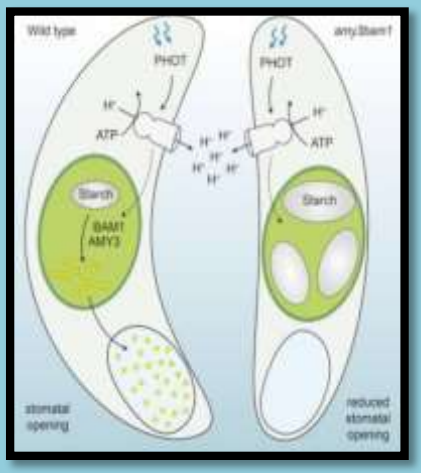



**Figure 5 : Blue light induces the proton to be pumped out creating an electro-chemical gradient.**

# ROLE OF ZEAXANTHIN

•Zeaxanthin has a special controlling influence on the stomatal movements.

• Guard cell chloroplast have a specific blue light response because it contains zeaxanthin.

• The xanthophyll, zeaxanthin has been identified as a blue light photoreceptor in guard cells

• Zeaxanthin on the thylakoid membrane is corelaxed with different signal transduction molecules which in turn regulates $H^+$ - ATPase for proton pumping in the guard cells generating membrane potential for transport of $K^+$ and $Cl^-$ influx.

• Zeaxanthin mainly regulates the proton-potassium pump theory.

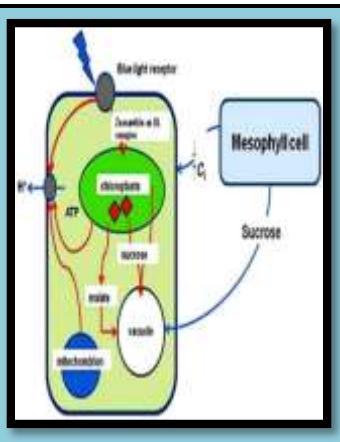



**Figure 6 : Zeaxanthin as a regulator of proton-potassium pump theory.**

## ROLE OF CARBON DIOXIDE

•Carbon dioxide($CO_2$) concentration in the vicinity of guard cells appears to be the most important key to stomatal mechanism.

•In high $CO_2$ concentration, stomata can be induced to close even in presence of light and can be open even in dark in low $CO_2$ concentration.

•If the leaf's internal concentration of $CO_2$ increases, the stomata are signaled to close because respiration is releasing more $CO_2$ than photosynthesis is using.There is no need to keep the stomata open and lose water if photosynthesis is not functioning.

• Alternatively, if the leaf's $CO_2$ concentration is low, stomata stay open to continue fueling photosynthesis.

## ROLE OF ABSCISIC ACID

• A high concentration of Abscisic acid(ABA) around the guard cells causes stomatal closure. Thus ABA is considered as a long range chemical signal for drought-induced stomatal closure.

• ABA causes stomatal closure by regulating ion channels in the plasma membrane.

• It induces influx of $H^+$ and efflux of $K^+$ in guard cells.

• The result is consistent with ABA functions in the inhibition of stomatal opening and induction of stomatal closure.
• So it can be considered as the inhibitor for the proton-potassium exchange pump theory.

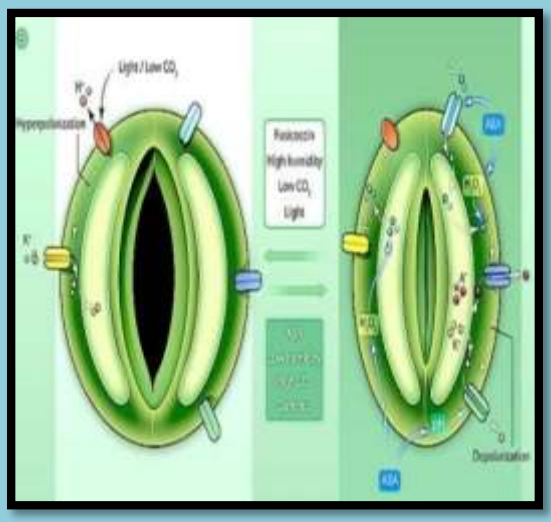
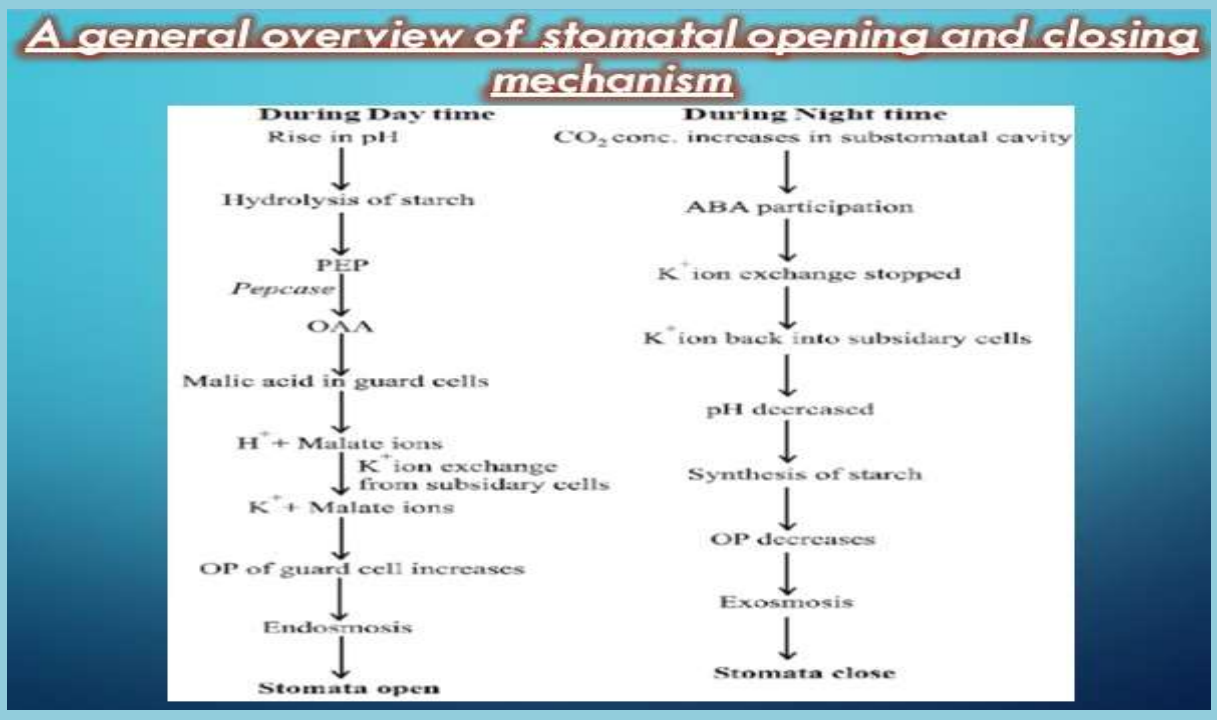


**Figure 7 : The inhibitory effect of ABA in stomatal opening.**




# Conclusion

Stomata are structural features of most of the plants. It plays a vital role in plant physiology as the excess water of plant body exit through the

stomata. After the discovery of the theories regarding stomatal opening and closing mechanism, it becomes easier to understand. The driving force for stomatal movement is turgor pressure. To conclude the topic the immense effect of blue light , zeaxanthin, aba, carbon dioxide can be added.


# Acknowledgement

I would like to express my sincere gratitude to Dr. Tripti Roy and Dr. Debjani Sinha Ray for providing me an opportunity to do such a presentation on this wonderful topic. I am also grateful to them for their continuous support and guidance in this regard. Finally, I sincerely thank my fellow classmates for their support and keeping patience during my presentation.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# Paper-XXI

# Groundwater and Its Management In India

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# Abstract

Groundwater is the most preferred source of water in various user sectors in India on account of its near universal availability, dependability and low capital cost. The increasing dependence on ground water as a reliable source of water has resulted in indiscriminate extraction in various parts of the country without due regard to the recharging capacities of aquifers and other environmental factors. There are areas in the country, where ground water development is suboptimal in spite of the availability of sufficient resources, and canal command areas suffering from problems of water logging and soil salinity due to the gradual rise in ground water levels. The development of ground water in the country is highly uneven and shows considerable variations from place to place. Though the overall stage of ground water development is about 58%, the average stage of ground water development in North Western Plain States is much higher (98%) when compared to the Eastern Plain States (43%) and Central Plain States (42%). Management of ground water resources in the Indian context is an extremely complex proposition. The highly uneven distribution and its utilization make it impossible to have single management strategy for the country as a whole. Any strategy for scientific management of ground water resources should involve a combination of supply side and demand side measures depending on the regional setting.

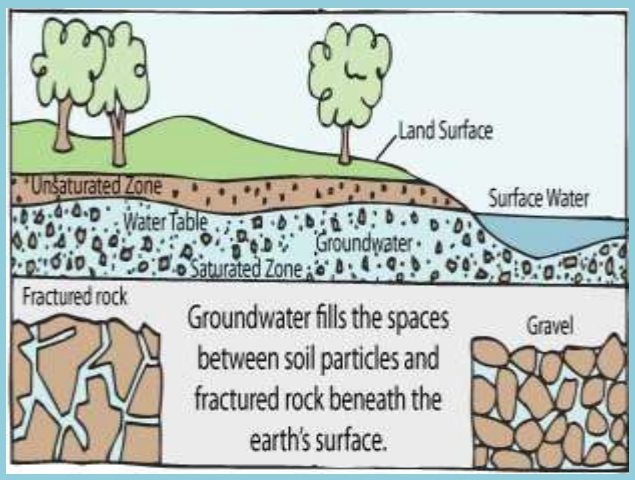
# Introduction

Groundwater is the water present beneath Earth's surface in rock and soil pore spaces and in the fractures of rocks.

On the Earth, approximately 3% of water is fresh water. Of this groundwater comprises 95%, Surface water 3.5% and soil moisture

1.5%. Out of all the fresh water available on earth, only 0.36% is available for use.

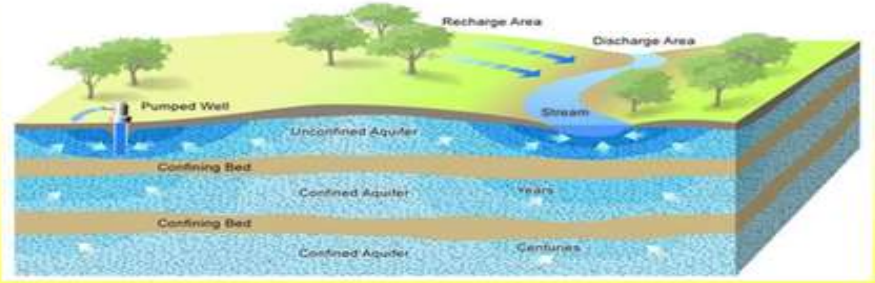
(Leopold,1974)




| SURFACE WATER | GROUNDWATER |
|---|---|
| Surface water is the water that is above the surface of the Earth. | Groundwater is the water that occurs below the surface of the Earth. |
| Found at permanent sources such as oceans, semi permanent sources such as some lakes , and man made sources such as reservoirs. | Found at groundwater table, aquifers, soil pore spaces and rock fracture points. |

## LET'S KNOW SOME COMMON TERMS:

- **AQUIFER:** An aquifer is an underground layer of water bearing permeable rock, rock fractures or unconsolidated materials from where groundwater can be extracted.



## Other two common terms:

- **Water table:** The upper surface of zone of saturation where pores and fractures are saturated with water.
- **Floodplain:** Flat land area adjacent to a stream composed of secondary deposits and subject to periodic inundation by stream.


## USES OF GROUNDWATER:

- As per the latest assessment, the annual replenishable groundwater resource of our country is 433 billion cubic meters, out of which 399 billion cubic meters is available for various uses.
- It is used for 85% of India's rural domestic requirements
- For 50% of urban water requirements
- More than 50% for irrigation requirements

# HYDROGEOLOGICAL SETUP OF INDIA

- **<u>Northern Mountainous Terrain and Hilly areas:</u> Kashmir to Arunachal Pradesh**

**Yield Potential Range 1-40 litre per second.**

**Rich source of recharge for Indo Gangetic and Brahmaputra alluvial plains.**

- **<u>Indo-Gangetic Brahmaputra alluvial plain:</u> Punjab, Haryana, Uttar Pradesh, Bihar, Assam, West Bengal**

**Yield Potential Range 25-50 litre per second.**

**Potential and productive groundwater reservoir and multi aquifer system**

- **<u>Peninsular shield area:</u> Karnataka,Maharashtra,Tamil Nadu,Andhra Pradesh, Orissa, Kerala**

**Yield Potential Range 2-10 litre per second.**

**Limited groundwater potential due to weathered rocks**

- <u>Coastal area:</u> **Gujarat, Kerala, Tamil Nadu, Andhra Pradesh and Orissa**

**Yield Potential Range 5-25 litre per second.**

**Development of aquifers entail saline water ingress**

- **<u>Cenozoic fault basin and low rainfall areas:</u> Arid and semi arid tracts of Rajasthan and Gujarat**

**Yield Potential range 1-10 litre per second.**

**Scanty rains and groundwater occurrence is restricted to deep aquifer systems.**


# DECLINING LEVEL OF GROUND WATER:

**Water table declined by 61% in the last decade in India.**

# GROUNDWATER MANAGEMENT STRATEGIES

## LET'S PRESERVE IT BEFORE IT'S TOO LATE

❖ **Suitable areas for groundwater development program:**

***Coastal areas:** Groundwater development should be done with caution to prevent saline water intrusion. Large diameter dug wells, filter point wells, shallow tube wells, infiltration galleries can be constructed.

***Water logged areas**: Good scope for further ground water development as shallow water table could be lowered down to 6 metres or more.

***Flood plain aquifers:** Flood plains of rivers are good repositories of ground water.

❖ **Rainwater harvesting and artificial recharge:** Implemented by Central Ground Water Board and other organizations. Tidal regulators has been constructed in coastal areas to impound fresh water upstream. Modification of natural movement of surface water into aquifers through check dams, percolation ponds, recharge pits in rural areas. Roof top rainwater harvesting is suited for urban areas.

❖ **Demand side measures:** Ownership of groundwater, need based allocation and pricing of resources, involvement of stakeholders in planning, execution and monitoring of projects and effective implementation of regulatory measures are the important considerations.

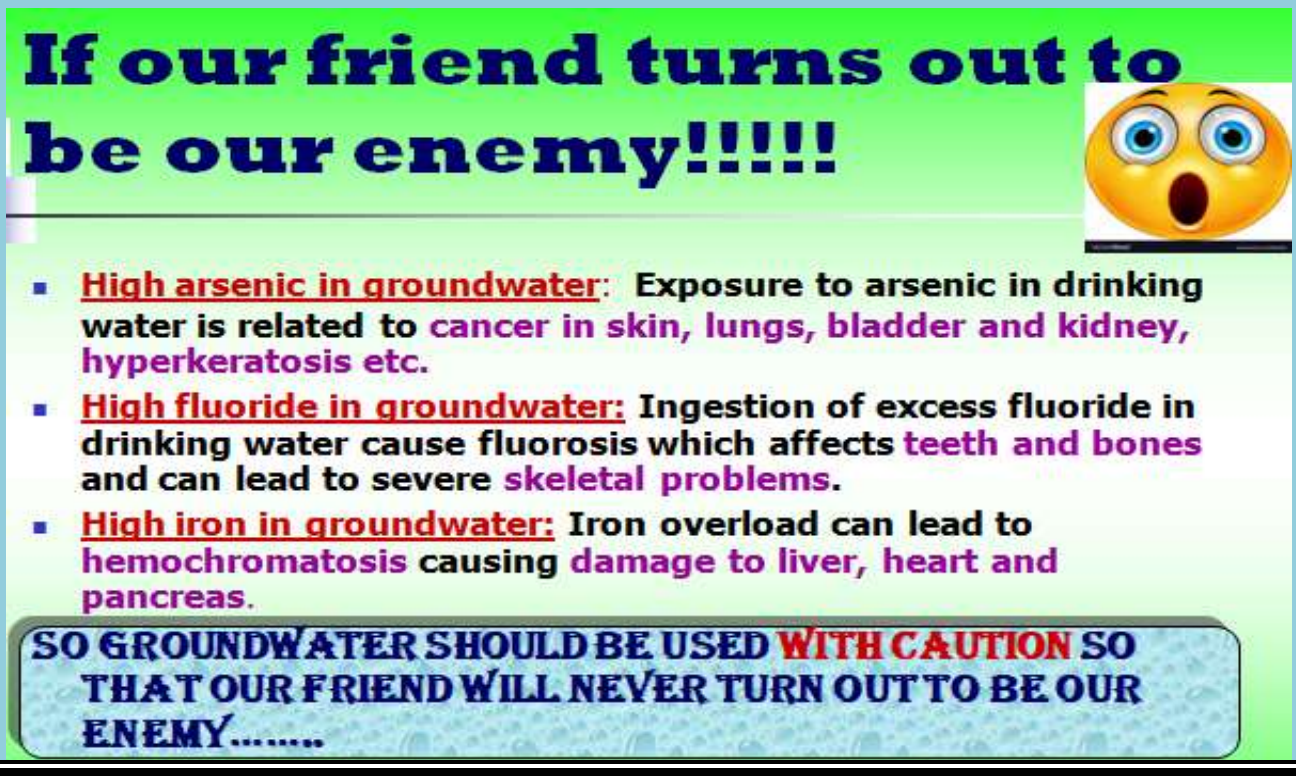
❖ **Regulation of groundwater development:** Various regulatory bodies take major role for sustainable use of ground water:
The Central Ground Water Authority, constituted under Environment protection Act (1986)

**Planning Commision 2007**

❖ **Management measures**: ***Cultivation of those crops which require less irrigation in ground water depleted areas.**

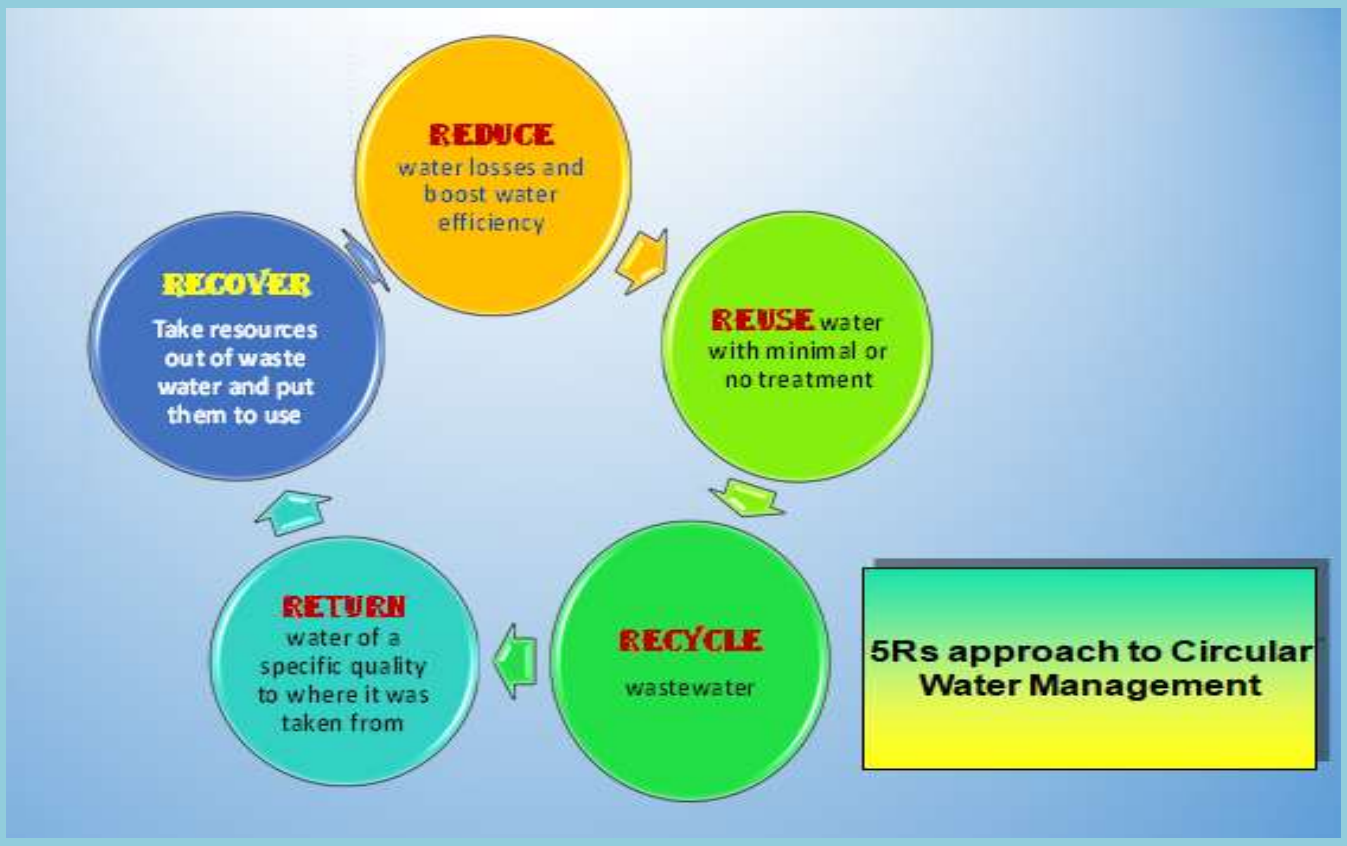
***Canal water should be supplied or well fields may be developed in outskirts of cities and water be supplied through pipeline.**

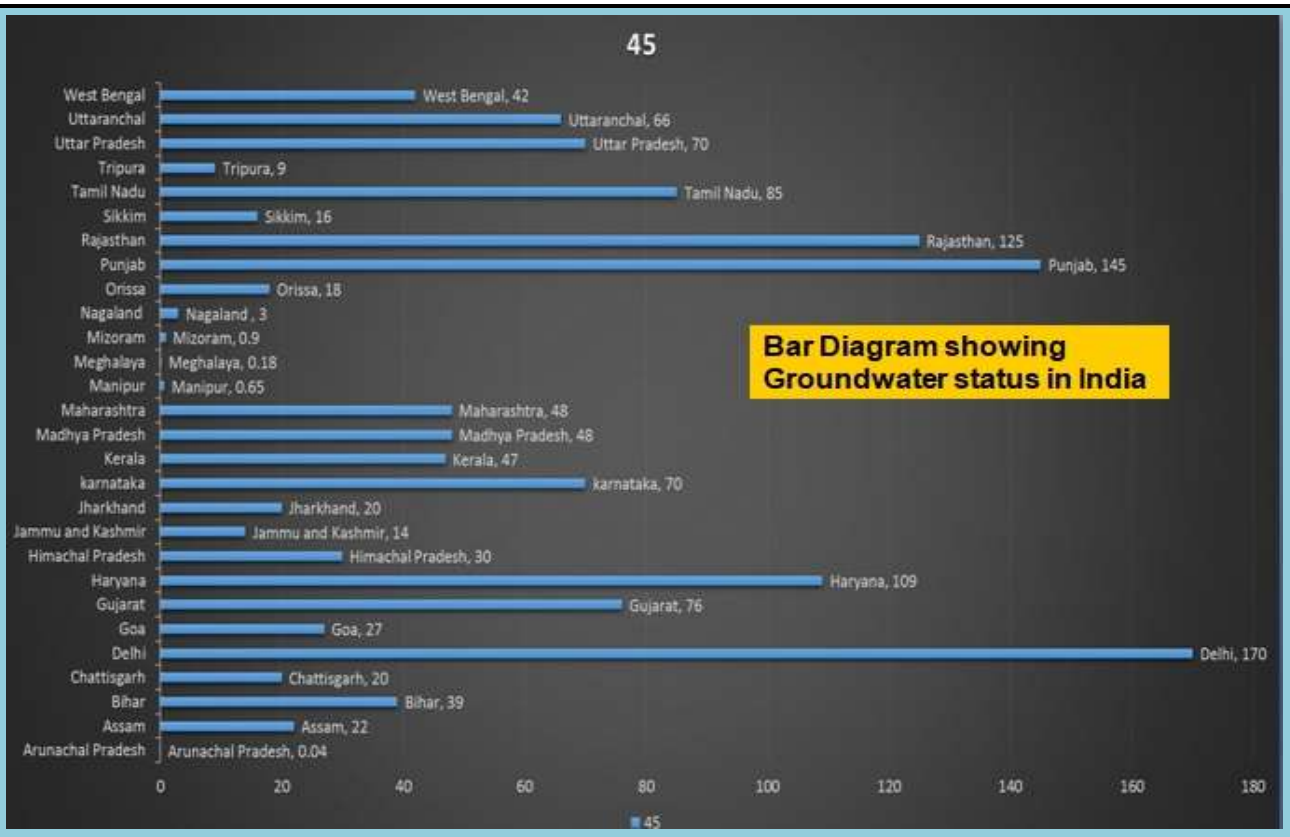
❖ **Groundwater pollution should be under control: The significant sources are agricultural chemicals, septic wastes, hazardous chemicals, atmospheric pollutants, underground pipes etc. We should be cautious otherwise our water for life will be lost forever.**

# FUTURE ACTION PLAN FOR GROUND WATER DEVELOPMENT IN INDIA:

- Rehabilitation, maintenance and construction of public tube wells
- Renovation of dug wells with geogenic contamination
- Artificial recharge schemes in marginal alluvium and piedmont areas
- Estimation of evapotranspiration loss
- Groundwater ecology of water bodies
- Reclaiming water logged areas by ground water abstraction.






# Conclusion

The highly diversified hydrogeologic settings and variations in the availability of ground water resources from one part of the country to other call for a holistic approach in evolving suitable management strategies. The emphasis on management needs does not imply that ground water resources in India are fully developed. Effective management of available ground water resources requires an integrated approach, combining both supply side and demand side measures. There is a vast area in the Indo Gangetic alluvial plain where the ground water development is sub optimal and there is sufficient scope for future development. Similarly, urgent action is required to augment the ground water in the water stressed areas. However, focus on development activities must now be balanced by management mechanisms to achieve a sustainable utilization of ground water resources. Ground water constitutes the most important source of irrigation water in the Gangetic

plains including the three states i.e. Bihar, Punjab and West Bengal. The productivity in terms of agricultural output is relatively low in Bihar and West Bengal as compared Punjab. Though, groundwater development for irrigation is feasible in these areas based on hydrogeological and environmental considerations , there is often a great economic barrier for the predominantly small and marginal farmers . A multitude of mechanisms have been developed or have emerged in these areas to enable farmers to benefit from ground water. Assured power supply is one of the key factors, the tariff, access and availability of which to a large extent determines the ground water use. Since the ground water development is mostly demand driven, it can be geared up through proper agricultural, credits, subsidy and energy support policies along with creation of suitable markets. In addition, the flood plains along the major river courses of the country offer good scope for groundwater development. Similarly, there are areas in the country with artesian condition, which can be mapped and suitable development plans formulated. In the alluvial areas, where multi-aquifer systems exist, there is a need to concretize methodologies for assessment of development potential of deeper aquifers. There is urgent an need for coordinated efforts from various Central and State Government agencies, non-Governmental and social service organizations, academic institutions and the stakeholders for evolving and implementing suitable ground water management strategies in the country.

# Acknowledgement

I would like to express my special thanks and gratitude to my respected teacher **Dr.Tripti Roy** who has provided me the golden opportunity to do this wonderful presentation. I came across so many new things. I am also thankful to my respected teacher **Dr.Debjani**

**Sinha Roy** . Without their continuous support and encouragement it was not possible for me to prepare this presentation. They have provided me constant support each and every day. I am also thankful to my respected teacher **Dr.Seemanti Ghosh** who has enriched me with her vast knowledge on this topic. I am grateful to all my respected teachers of Botany department, Bethune College. Last but not the least I am always thankful to my parents and friends for their enormous help and support.

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# Paper-XXII

# BIOCHEMISTRY OF NITROGEN FIXATION WITH SPECIAL EMPHASIS ON DINITROGENASE AND LEGHAEMOGLOBIN

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# Abstract

Dinirtrogen is not easily reduced because the interatomic nitrogen bond (triple bond)is very stable .In the industrial process ,reduction of the dinitrogen triple bond with hydrogen can be achieved only at high temperature and pressure and at the cost of considerable energy .Biological reduction of dinirtrogen is equally costly ,consuming a large proportion of the photoassimilate provided by the host plant .

# Introduction

▶ Nitrogen fixation is the process of conversion of free nitrogen into organic forms to make it available for plants . It is the single most important method which meets the nitrogen demands for plants.

▶ Biological nitrogen fixation is of two types :

i)symbiotic nitrogen fixation : Symbiotic associations are formed with higher plants in which the prokaryotes directly provides the host plant with fixed nitrogen in exchange for nutrients. Symbioses occur in nodules of the host plant .Example: Legumes – Rhizobia ,*Azolla*-*Anabaena* , *Parasponia*- *Bradyrhizobium etc*.

ii)Free living or non symbiotic nitrogen fixation:

▶ Prokaryotes such as *Bacillus* , *Klebseilla* etc live in the soil independently of other organisms.

▶ Prokaryotes mostly accounts for the fixation of atmospheric nitrogen into ammonium , and thus representing the key entry point of molecular nitrogen into the biogeochemical cycling of nitrogen .

▶ Most of the Plants obtain nitrogen from soil either in the form of nitrate($NH_3^-$) or ammonia ($NH_4^+$)

▶ Nitrogen fixation is a prokaryotic domain, because only prokaryotic organisms have the enzyme complex , called dinitrogenase, that catalyzes the reduction of dinitrogen to ammonia.

▶ It is a complex biochemical and physiological process.

▶ Prokaryotes that fix dinitrogen are called nitrogen fixer.

## BIOCHEMISTRY OF NITROGEN FIXATION

### STRUCTURE OF DINITROGENASE

- Dinitrogenase is a multimeric protein complex
- Contain two protein components

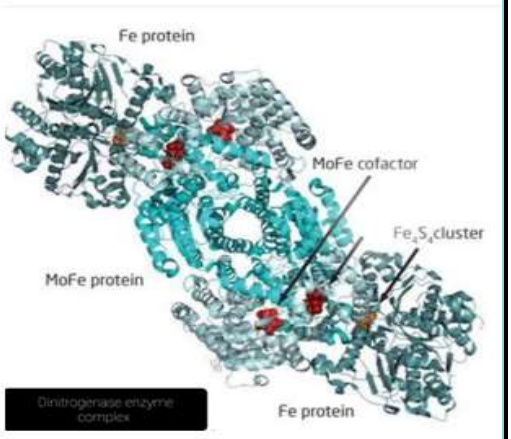
i)Iron (Fe) protein:

- smaller of two components.
- dimer i.e. two identical subunits(vary in mass 30 -72 kDa depending on the bacterial species)
- each subunit contains iron –sulphur cluster (4Fe and 4 $S^{2-}$)

ii)Molybdenum Iron (MoFe)protein:

(total molecular mass of 180 to 235kDa ,depending on the bacterial species )

- Tetramer i.e. It has four subunits
- Each subunit has two Mo-Fe-S clusters.




3D structure of dinitrogenase enzyme complex

### MECHANISM :-

- In the overall reduction reaction ,ferredoxin(Fd) serves as an electron donor to the Fe protein , which in turn binds and hydrolyzes ATP Adenosine triphosphate to the Fe protein are thought to cause a conformational change of the Fe protein that facilitate the redox reactions.
- The Fe protein reduces the MoFe protein
- And the MoFe protein can reduce substrates , although under natural conditions ,it reacts only with $N_2$ and $H^+$.
- Under natural conditions , substantial amounts of $H^+$ are reduced to $H_2$ gas ,and this process can complete with $N_2$ reduction for electrons from dinitrogenase.
- In some rhizobia , hydrogenase ,an enzyme that can split the $H_2$ formed and generate electrons for $N_2$ reduction , thus improving the efficiency of nitrogen fixation .

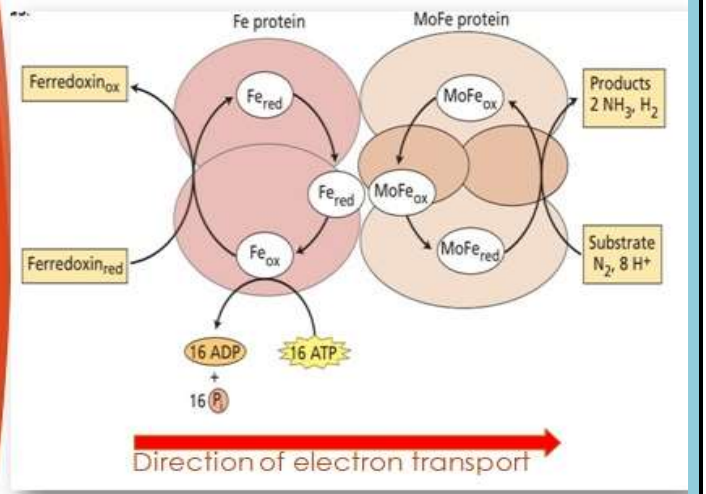


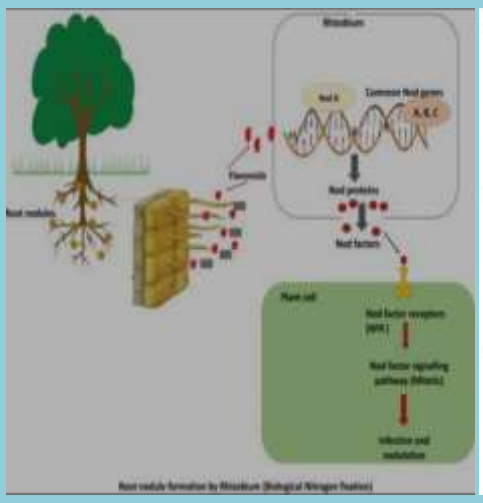

Fig : DINITROGENASE ENZYME complex

- Biological nitrogen fixation ,produces ammonia from molecular nitrogen . The overall reaction is :-

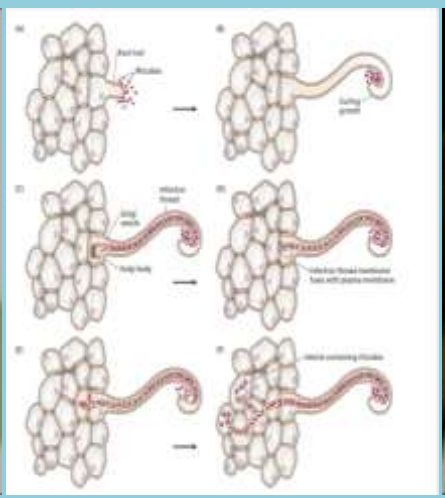
$$N_2 + 8\ e^- + 8\ H^+ + 16\ ATP \rightarrow 2\ NH_3 + H_2 + 16\ ADP + 16\ P_i$$

- The reduction of $N_2$ to $2NH_3$,a six –electron transfer , is coupled to the reduction of two protons to evolve $H_2$. This reaction is catalyzed by nitrogenase enzyme complex .

# NODULE FORMATION IN BRIEF :



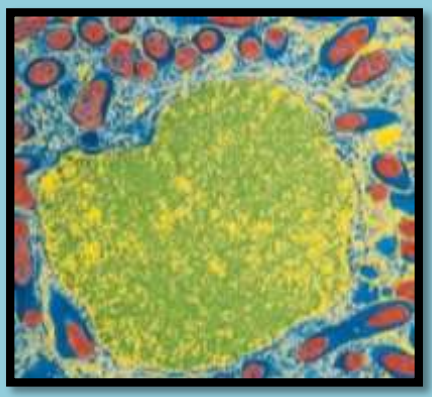
NOD factors produced by bacteria act as signals for symbiosis.



Root nodule formation involve infection and nodule organogenesis

Nodule

Nitrogen fixing nodules on roots of *Pisum sativum*



Electron micrograph of pea root nodule
Bacteroids (red) inside the root nodule cells surrounded by the peribacteroid membrane (in blue)

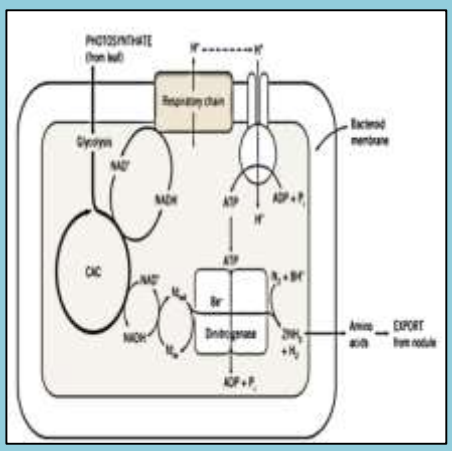



**FIGURE : DIAGRAM ILLUSTRATING THE INTERACTIONS BETWEEN PHOTOSYNTHESIS,RESPIRATION AND NITROGEN FIXATION IN BACTERIODS**

# DINITROGENASE IS SENSITIVE TO OXYGEN

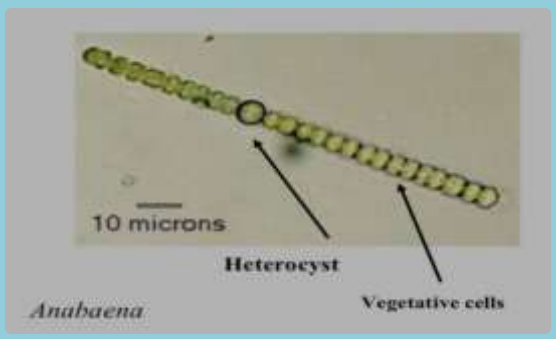
-One of the most crucial problems facing nitrogen-fixing organisms is the sensitivity of dinitrogenase to molecular oxygen .

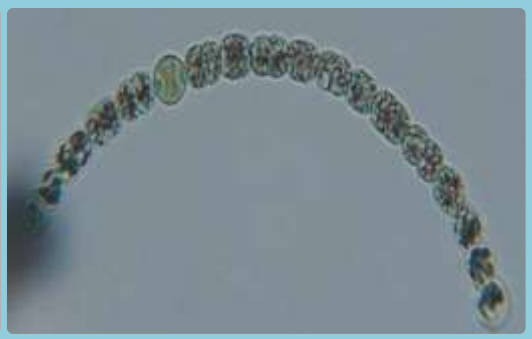
-Both the Fe protein and the MoFe protein are rapidly and irreversibly inactivated by molecular oxygen .

-Several strategies for regulating oxygen level have developed to resolve this conflict.

**FIRST**, many free living bacterial nitrogen fixers have retained an anaerobic lifestyle or if facultative , fix dinitrogen only under anaerobic conditions .production of ATP is less efficient under anaerobic conditions , which may offer a partial explanation for ,why in spite of their large numbers , free living nitrogen fixers contribute a relatively small proportion of the total nitrogen fixed biologically.

**SECOND**, certain species of nitrogen – fixing cyanobacteria such has Nostoc and Anabaena have structurally isolated nitrogen fixing apparatus called heterocysts . Heterocysts are exclusive sites for nitrogen fixation. Heterocysts have thickened ,multilayered cell walls that restrict diffusion of oxygen. Moreover , heterocysts are photosynthetic cells ,they lack photosystem II and thus do not evolve oxygen .



## HETEROCYSTS

NITROGEN FIXATION IS CARRIED OUT IN THE ENLARGED CELLS OR HETEROCYSTS, WHOSE STRUCTURE AND METABOLISM LIMITS THE CONCENTRATION OF FREE OXYGEN.

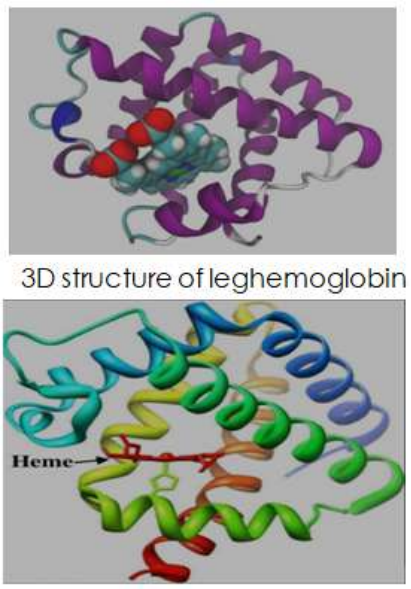
**THIRD** , the oxygen supply is regulated to a large extend by an oxygen binding protein called leghemoglobin in legume nodules.

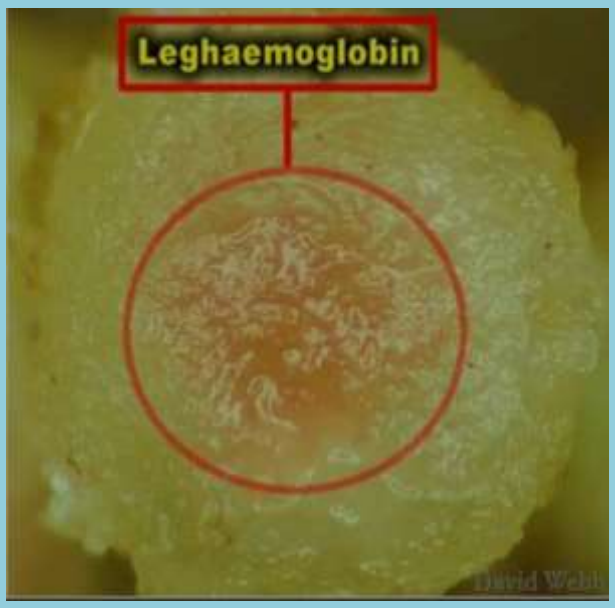
Leghemoglobin is present in the cytoplasm of infected nodule cells and gives the nodules a pink colour.

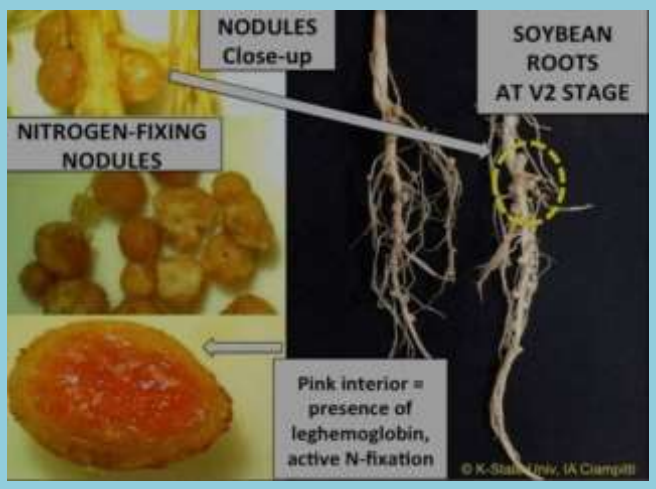
The host plant produces the globin portion whereas bacterial symbiont produces the heme portion.

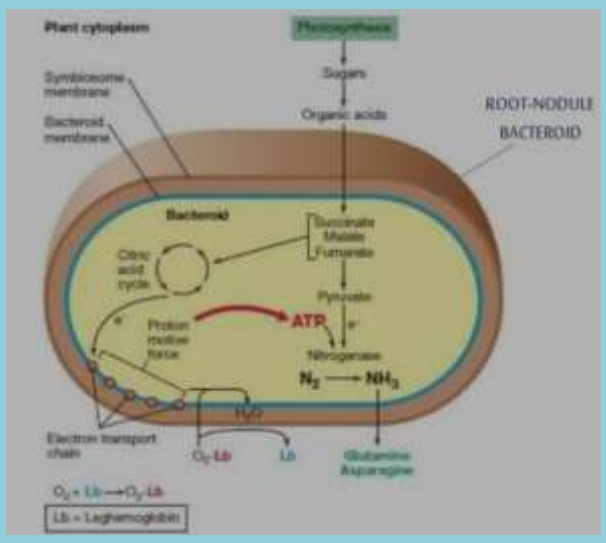
The equilibrium concentration of oxygen in bacteroid zone is thus kept at a level sufficient to support bacteroid respiration –and the production of ATP and reducing potential –while at the same time preventing excess oxygen from inactivating dinitrogenase.

Oxygen level must be carefully balanced ,because too low an oxygen concentration can also limit dinitrogenase activity in nodules. This could be result of limiting ATP availability .



# Conclusion

Nitrogen fixation is the process of conversion of free nitrogen into organic form to make it available for plants .It is may be of two types - Symbiotic nitrogen fixation and free-living or non -symbiotic nitrogen fixation.Biological nitrogen fixation accounts for most of the fixation of atmospheric nitrogen into ammonium,and thus representing the key entry point of molecular nitrogen into the biogeochemical cycle of nitrogen .

# Acknowledgement

I would like to thanks my departmental teachers for providing this golden opportunity of giving a presentation on the topic : biochemistry of nitrogen fixation with special emphasis on dinitrogenase and leghemoglobin. And special thanks to Dr.Tripti Roy and Dr.Debjani Sinha Roy for their constant support and guidance. And thanks to my fellow classmates for their support.

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Paper-XXIII

# Glycolysis In Plants

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# Abstract

Glycolysis is a process through which glucose is broken down producing energy in the form of ATP. It generally occurred in cytoplasm of the cell. It generally occurred through two phases like preparatory phase and pay off phase.

In this topic I have mentioned on the basic material like definition, site of occurrence and reaction involved in glycolysis process.

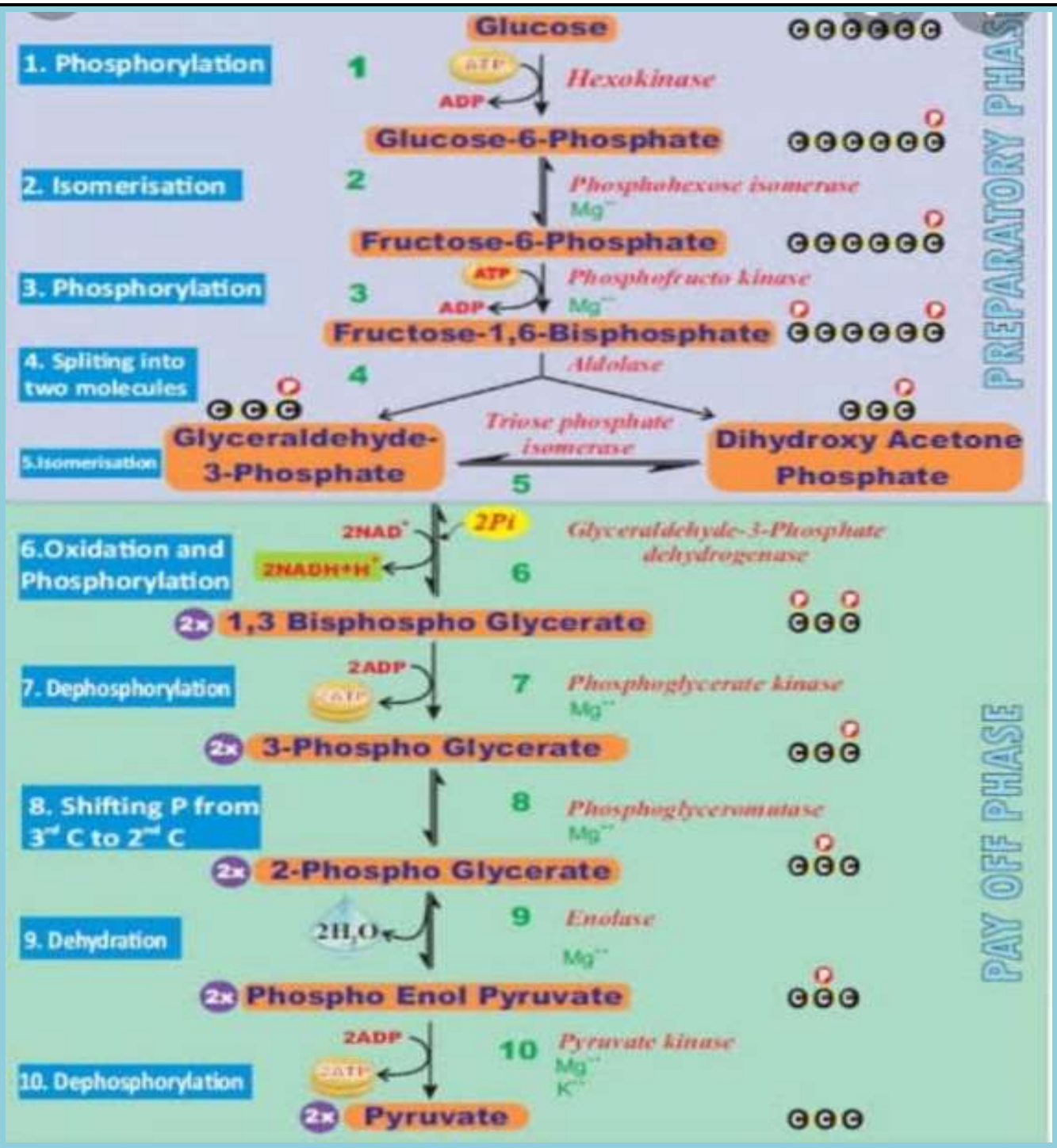
# Introduction

Glycolysis is a Greek word. Glykos means sugar and lysis means splitting. This process is also known as EMP pathway.
The full form of EMP is Embden-Meyerhof-Parnas which is discovered by Gustav Embden, Otto Meyerhof and Jakub Karol Parnas.

Glycolysis is the process in which glucose is broken down to produce energy. It produces two molecules of pyruvate, ATP, NADH and water.

## WHERE DOSE EMP PATHWAY OCCUR?

Glycolysis is the process which occur in cytoplasm of cell. It does not use oxygen. There is a net gain of two ATP molecules.




# Conclusion

Overall, Glycolysis converts one molecule of glucose to two molecules of pyruvate acid. For other to begin it needs two ATPs. The end product which is pyruvate acid may enter the Krebs cycle is oxygen is available, and if oxygen isn't presented it creates a fermentation pathway.

# Acknowledgement

I would like to express my special thanks and gratitude to Dr. Tripti Roy and Dr. Debjani Sinha Roy for giving me the golden opportunity to present my topic and I would convey my thanks to my fellow classmates for cooperating me and guiding me.

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# Paper-XXIV

# Allosteric enzymes: Their regulation mechanism and Behaviour

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# Abstract

Allosteric enzymes are multimers that show regulatory activity by having a binding and a regulatory site. They are of two types based on the modification type -Homotropic(substrate itself is a modulator) and Heterotropic(modulator is other than the substrate). Again modulator is of two types,i.e. positive and negative modulator.Allosteric enzymes show sigmoid kinetics which is the hallmark for "cooperativity".The heterotropic allosteric enzymes show K-type and V-type activity curves.The substrate binding to the allosteric enzymes show cooperate behaviour ,one bound substrate increases the affinity for other subsequent binding of substrates .Enzymes exist in two conformations -Tense or T state and Relaxed or R state.There are two proposed models to demonstrate allosteric behaviour -Concerted model and Sequential model.Aspartate Transcarbamoylase (ATCase) is an allosteric enzyme responsible in the biosynthesis of pyrimidine nitrogenous base such as CTP. ATP and CTP is the positive and negative regulator of ATCase respectively.As CTP concentration increases it goes back and bind to ATCsae and inhibit its activity ,this is called feedback inhibition.The quartenary structure of ATCase is composed of two catalytic trimers ,each trimer made up of three identical polypeptide chain ,and three regulatory dimers,each dimer consists of two identical polypeptide chain.There are certain changes in the quartenary structure prevailing to the allosteric behaviour of ATCase .At low substrate concentrration the enzyme remains constrained and compact and as substrate increases the subunits move farther away and seems to expand .

# Introduction

"Allos" means "other" and steric means place or sites.

They are a class of regulatory enzymes which increase or decrease catalytic activities in response to certain signals .

They are placed at the beginning of a metabolic path way .They generally catalyze the irreversible reaction or a committed step in the pathway .

They physically and chemically differ from non regulatory enzymes by having two functionally different binding sites or subunits( generally polypeptide chains) called:

a)Regulatory site-where effectors bind

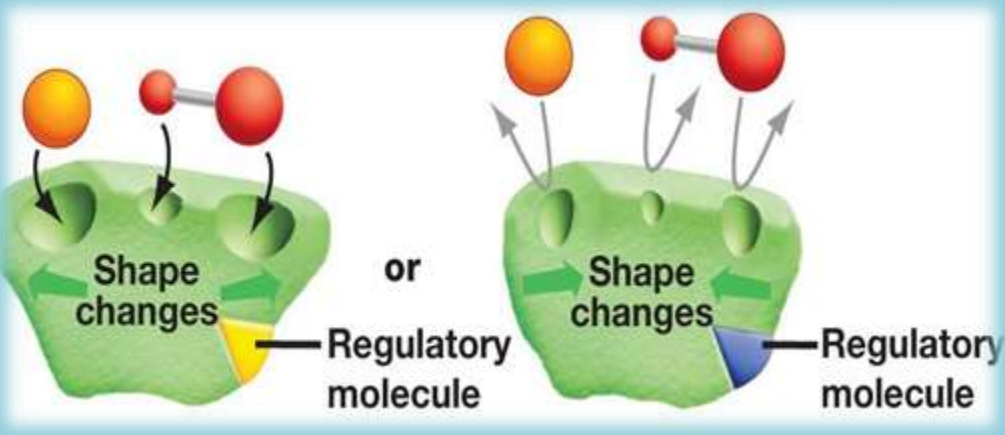
b)Active sites or catalytic sites -where substrates bind and catalyze the reaction.

They are generally larger than non regulatory enzymes having many active sites or multiple subunits ,thus they are oligomeric.

BASED ON THE MODIFICATION REGULATORY ENZYMES CAN BE:-

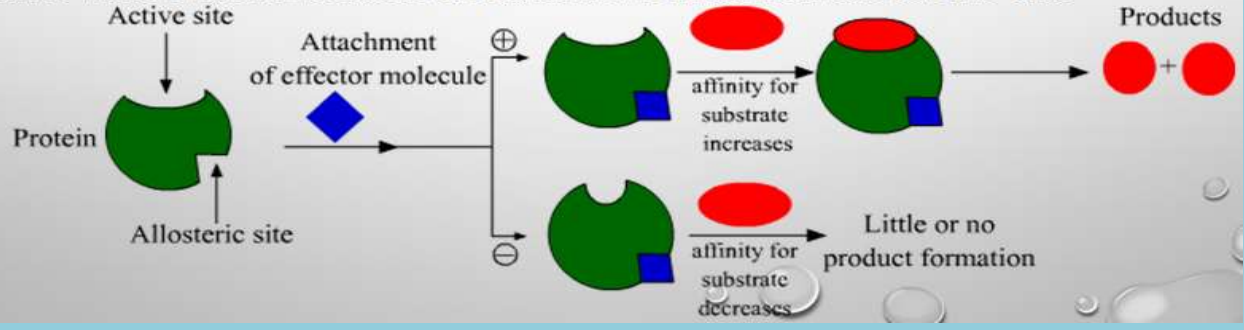
HOMOTROPIC –The modulator is the substrate itself or the modulator is chemically and physically similar to the substrate

HETEROTROPIC – The modulator is a molecule other than the substrate. Only the effector performs the role of regulation.

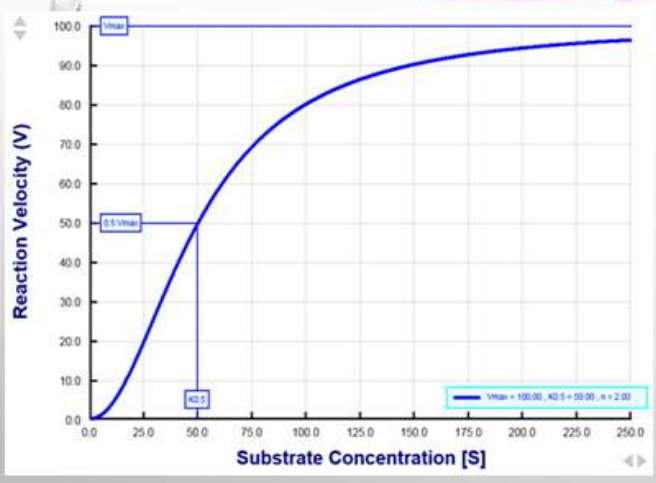


## MODULATORS ARE OF TWO TYPES:-

- **POSITIVE MODULATOR**:- THEY ARE ALSO CALLED ACTIVATORS BECAUSE THEY ACTUALLY BIND TO THE ALLOSTERIC SITE OF THE ENZYME AND ENHANCE THE ENZYME'S CATALYTIC ACTIVITY.
- NEGATIVE REGULATOR :- IF THE EFFECTOR MOLECULE BINDS TO THE ALLOSTERIC SITE OF THE ENZYME AND INACTIVATES THE ENZYME'S CATALYTIC ACTIVITY THEN THIS EFFECTOR MOLECULE IS KNOWN AS THE 'INHIBITOR'.
- ENZYMES WITH SEVERAL MODULATORS GENERALLY HAVE DIFFERENT SPECIFIC BINDING SITES




## Allosteric Enzyme show sigmoidal kinetics and don't obey MICHAELIS –MENTEN kinetics : Sigmoid curve is a hallmark for "cooperativity"




**Comparison of sigmoid graph with Michaelis –Menten hyperbola**

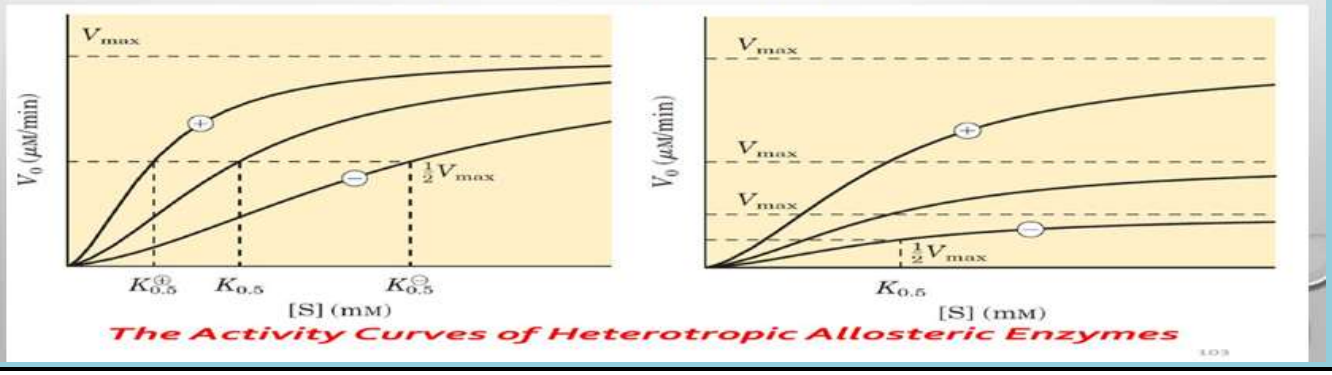
| *Hyperbolic curve* | *Sigmoidal curve* |
|---|---|
| • Shows for nonregulatory enzymes<br>• First order reaction<br>• Velocity gradually increases as substrate concentration increases<br>• Shows saturation effect of active sites<br>• Substrates bind independently to active sites | • Shows for allosteric enzymes<br>• Second order reaction<br>• Velocity abruptly increases twice as substrate concentration increases<br>• Shows saturation effect of active sites<br>• One binding of substrate increases the likelihood of binding other substrates to the unoccupied active sites . |

## BASED ON THE ALTERATION OF ENZYME KINETICS: ALLOSTERIC ENZYMES ARE CLASSIFIED AS:-

**K –type- Allosteric modulators can change the affinity(Km) of enzyme towards substrate .Example – Phosphofructokinase.**

**V –type – Allosteric modulators can change the reaction velocity. Example –Acyl CoA Carboxylate**

THE ALLOSTERIC REGULATORS CAN BOTH WORK IN A POSITIVE AND NEGATIVE FASHION TO MODULATE THE AFFINITY AND REACTION VELOCITY :




*The Activity Curves of Heterotropic Allosteric Enzymes*

# BEHAVIOUR OF ALLOSTERIC ENZYMES:-

•"Cooperativity" means when the first substrate binds to an active site of allosteric enzyme it increases the affinity of second active site for the next substrate ,and second substrate further increases the affinity of the third etc., so the affinity for the last substrate molecule is many times greater than that of first.

•The "cooperative" interaction between protein subunits are well illustrated by a non-enzyme ;oxygen binding to Haemoglobin :-

•It can be positive (i.e. binding of ligand to one site *increases* affinity of other site for ligand binding) or negative (i.e. binding of ligand to one site *decreases* affinity of other site for ligand binding)

•Negative cooperativity is displayed by 2,3-bisphosphoglycerate (BPG), it reduces the oxygen affinity of haemoglobin by binding to deoxy-haemoglobin and stabilising it.

## TO UNDERSTAND THE COOPERATIVE BEHAVIOUR OF ALLOSTERIC ENZYMES TWO MODELS ARE GENERALLY USED:-

•CONCERTED MODEL- PROPOSED BY MONOD,WYMAN,CHANGEUX(1965)/mwc/*all or none model*

•POSTULATES-

•a) Allosteric proteins are oligomers composed of an even number of identical subunits.

•b)Each monomer in an oligomer can exist in two conformations labelled as T(tense or taut)and R(relaxed),which are in equilibrium .

•c) The binding affinities of ligand to these two conformations are different .

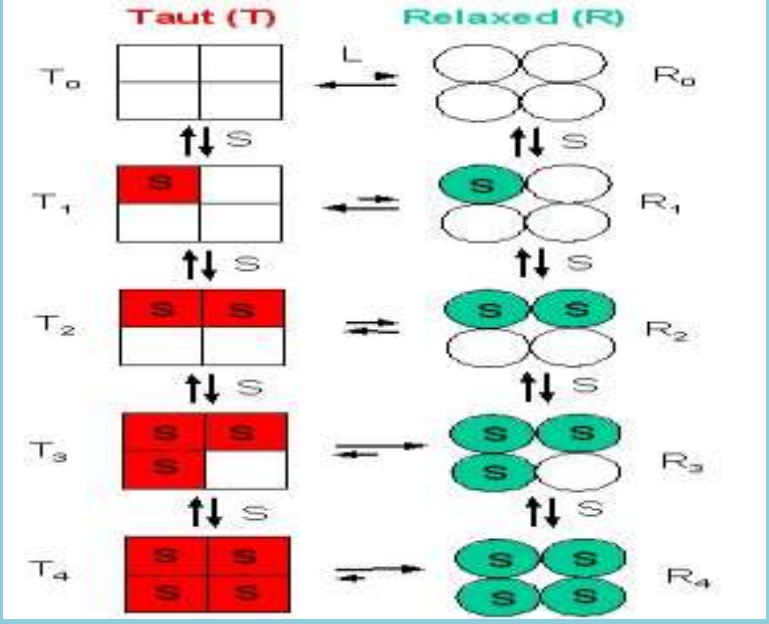
•d)At any given time all the monomers in an oligomeric molecule possess the same conformation.

# DISTINGUISHING FEATURE- CONFORMATION *OF ALL* SUBUNITS CHANGE SIMULTANEOUSLY

All subunits change conformation from the inactive T conformation to the active R conformation at the same time ; that is a concerted change of conformation occurs . The binding of the first substrate to one subunit facilitates the binding of second substrate molecule to other subunit .this is exactly what is meant by cooperative binding.

PROBLEM- According to this model, the affinity of other active site only increase when the enzyme tetrameric assumes the R state and this is in contrast what we observe experimentally.

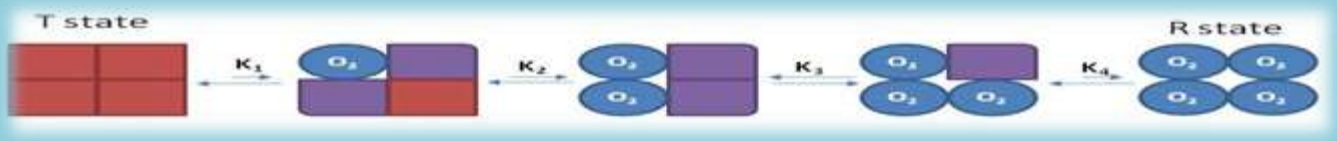
**WHY DO WE NEED TWO DIFFERENT MODELS TO DESCRIBE A SINGLE PHENOMENON IN NATURE?**




# SEQUENTIAL MODEL : PROPOSED BY KOSHLAND, NEMETHY, FILMAN (K N F)

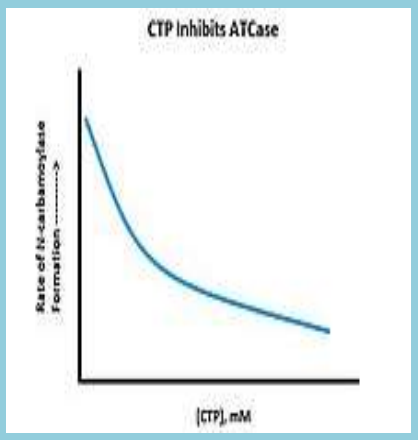
•Unlike concerted model ,the sequential model can have enzyme subunits( tetramers) that consist of both R and T state. According to this model as soon as a ligand binds to one subunit (polypeptide chain) ,there is a conformational change in that polypeptide subunit , which then induces a conformational changes in shape slightly of the other adjacent active sites.

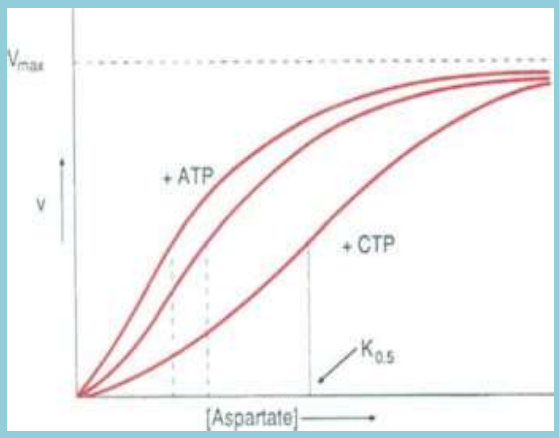
•Hence the effect of ligand binding is *sequentially transmitted through the interface between subunits producing increased or decreased affinity for ligand by contiguous protomers.*




## ATCase REGULATION AND FEEDBACK INHIBITION :-

Aspartate Transcarbamoylase (ATCase) is a classical example of allosteric enzyme . ATCase catalyzes the formation of N-Carbamoylaspartate from Aspartate and Carbamoyl Phosphate. This is the first step in the biochemical synthesis of pyrimidine nitrogenous bases that are ultimately used to produce nucleoside triphosphates such as Cytidine Triphosphate (CTP). Early studies demonstrated that the rate of formation of N-Carbamoylaspartate decreased as the concentration of the CTP increased inside the cell. this data suggested that CTP was an inhibitor to ATCase.

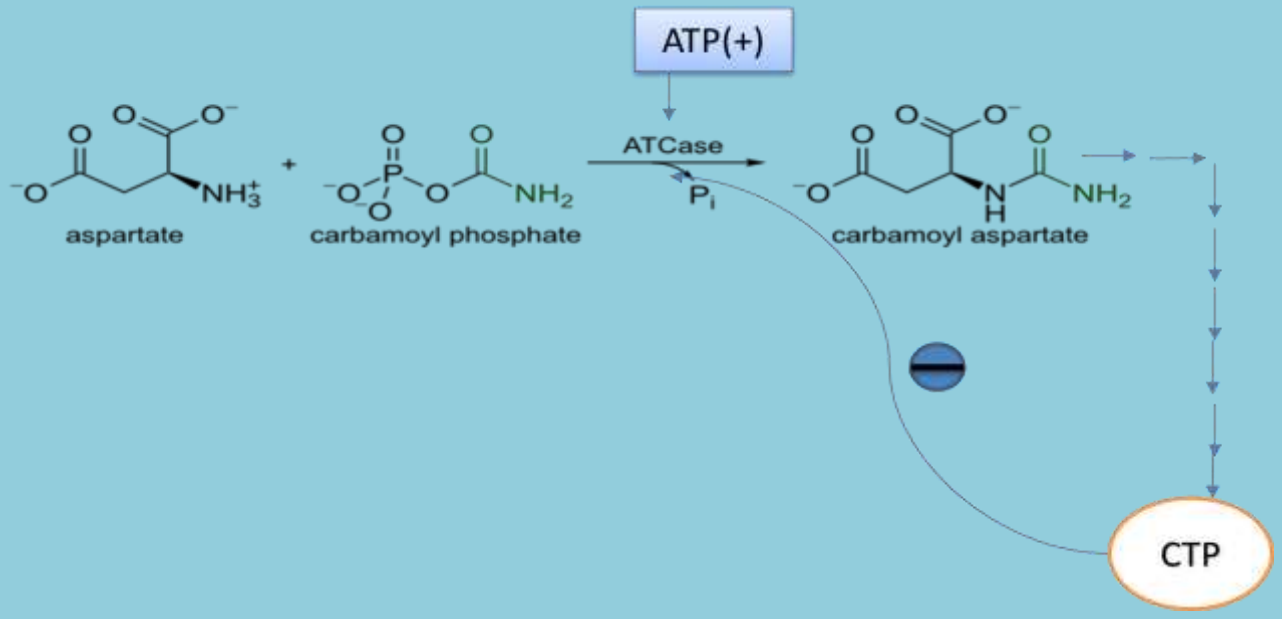


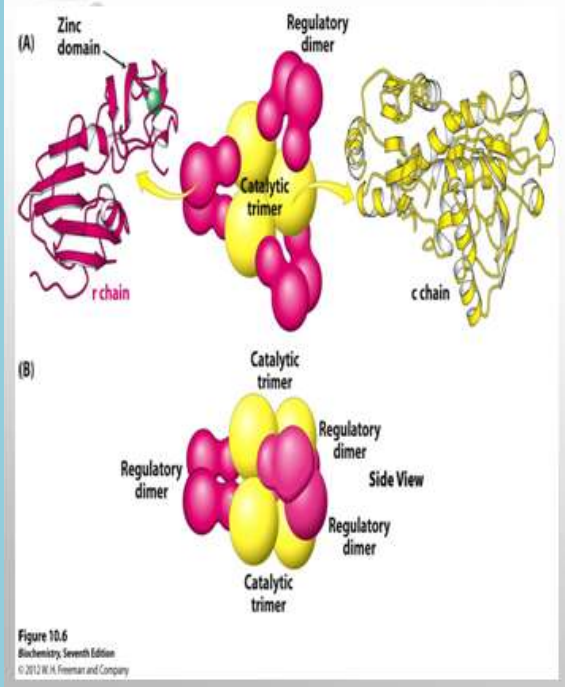

•These results suggest that the end product of the ATCase – initiated reaction must bind onto and inhibit the activity of ATCase .This is known as negative feedback inhibition .

•Since CTP looks nothing like the original substrate molecules of ATCase, that implies that CTP does not bind to the active site of the enzyme but rather some other regulatory site. therefore, that must mean that CTP is an allosteric inhibitor of ATCase and ATCase is in fact controlled allosterically by our cells.

- At low concentrations of CTP, not enough of it is present to inactivate the enzyme and so the enzyme will function efficiently to produce N-Carbamoylaspartate at a higher rate.
- However at high concentrations of CTP, the CTP creates a negative feedback loop that causes the inhibition of ATCase and blocks the formation of the product.

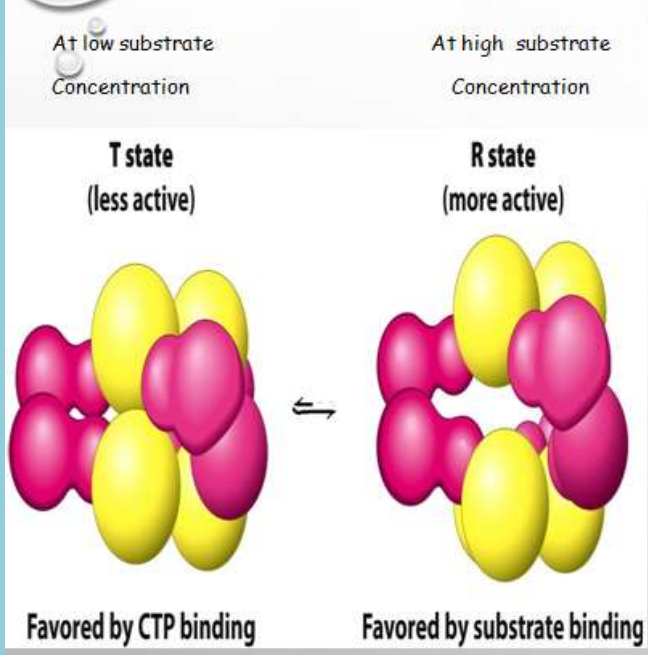



## QUATERNARY STRUCTURE OF ASPARTATE TRANS CARBAMOYLASE




- There are two catalytic trimers(C3).Each trimer consist of 3 individual and identical polypeptide C chain/ subunits .These respond to substrate but do not respond to CTP. The one catalytic chain is hidden beneath the other catalytic trimer.
- There are 3 regulatory dimers(r3).Each dimer consists of 2 identical r(regulatory) chain /subunits .These respond to CTP but not the substrate.
- Each r chain of the dimer interact with each of the catalytic chain in each of these trimers and this interaction is amplified by the presence of Zinc metal atom .
- Zinc atom is present at the interface of a r chain and a c chain. Each zinc atom is bound to four cystein residues.
- The whole quaternary structure is made up of 12 polypeptide chains- 6 catalytic chains and 6 regulatory chains.

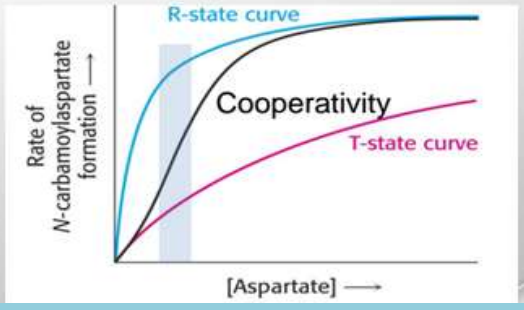
## STRUCTURAL CHANGES TO QUATERNARY STRUCTURE : SHOWS COOPERATIVITY




- At low substrate concentration, the quaternary structure of Aspartate Transcarbamoylase exists predominantly in its Tense (T) state. In the T-state, the two catalytic trimers are found in close proximity and so they create a very compact and constrained structure in which the active sites have a low affinity (high Km) for the substrate molecules and display a low catalytic activity.
- As the substrates are added ,it causes the two trimers to move farther apart and rotate ,which in turn causes the r chains to also move. Therefore the entire structure seems to expand upon substrate binding
- As the substrate concentration rises, the substrate molecules begin to bind to the active sites, which begins to shift the equilibrium towards the relaxed (R) state. As more and more substrate binds, the shift becomes more pronounced until eventually the R-state structure predominates. In the R-state, the two trimers have rotated and moved farther apart, which decreases the constrain in the quaternary structure. This in turn increase the active sites affinity (low Km )for the substrate and increases the activity of the enzyme. This type of behaviour is called cooperativity and the concerted model can be used to describe the mechanism by which it takes place

## ASPARTATE TRANS CARBAMOYLASE DEMONSTRATE SIGMOID CURVE

- Rink curve shows Michaelis-Menten curve as when the [S] is low the quaternary structure will exist predominantly in the T-state (high Km). Rate of activity will be low and that's because active sites have low affinity for that substrate
- But as we begin to increase the [S] ,that begins to shift the entire curve (equilibrium )to the R-state and once we transform the quaternary structure from the T state to R state , the blue curve begins to describe the quaternary behaviour .So , according this blue curve we see that at same [S] the rate of activity is much higher .
- If we combine these two curves we obtain the actual black curve for ATCase .This is we know as concerted model, i.e. all or nothing behaviour in which the enzyme either exist just in the T-state or in the R state in which the enzyme basically carries out the catalytic reaction.
- R state= ——
- T state= ——
- Actual sigmoidal curve For ATCase= ——

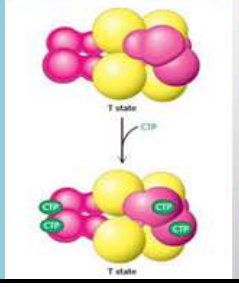



# REGULATORS OF ASPARTATE TRANS CARBAMOYLASE

**Two heterotropic effectors of ATCase are – CTP and ATP**

CTP forms the negative feedback inhibition loop and an allosteric inhibitor binds onto the regulatory dimers and decreases the ATCase activity .

CTP stabilizes the energy of the T-state and makes it much more stable and that shifts the equilibrium from the R-state into T-state. By binding onto the regulatory chains (r6) the CTP molecules makes it more difficult for the substrate to convert ATCase to R state.




*ATP is also an allosteric regulator of ATCase .It binds to the same site of regulatory chains but it actually increases the activity of the enzyme.

* At high concentration of ATP , the ATP will displace CTP and will help shift the equilibrium towards R state . As a result the catalytic activity of ATCase will increase.

* Physiologically ,a high ATP concentration means that there is an unequal distribution of nucleoside triphosphate (purine to pyrimidine) ,Therefore, the cell tends to equalise the distribution by producing more nucleoside triphosphate such as CTP.

# Conclusion

Allosteric enzymes are enzymes which posseess both regulatory and active sites.the enzymes are generally oligomeric. Regulatory sites are also called allosteric sites. They attract allosteric substances, modulators or effectors of two types- activator and inhibitor.These enzymes show characteristic sigmoid kinetics instead of routine Michelis-Menten kinetics . This sigmoid kinetics is explained by concerted model and sequential model . These models could only be understood if and only when the substrates binding occurs in a cooperative fashion .However , we could clearly see some limitation of concerted model which could be simply overcome by following sequential model. The allosteric enzymes exhibit reversible non-competitive inhibition.The inhibitors are generally products or intermediates of reactions catalyzed by the enzymes. Therefore, allosteric inhibition called end product inhibition or feedback inhibition.Feedback inhibition has a regulatory role in checking excess of product formation . The classic example of feedback inhibition is demonstrated by ATCase enzyme.Its regulatory role is attributed to its quartenary structure comprising of 12 polypeptide chains. CTP and ATP are positive and negative regulator of ATCase respctively.

# Acknowledgement

I deeply express my overwhelming gratitute towards Dr. Tripti Roy and Dr. Debjani Sinha Roy, associate professors of Bethune college for providing me this opportunity to put forward my

ideas and concepts on this topic. It was an exhilarating experience which I will surely put to good use in future. I sincerely thank my fellow batch mates for supporting me throughout this academic year.

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Paper-XXV

# Signaling Mechanisms with Respect to NODULATION

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# Abstract

The cycling of nitrogen between geochemical and biochemical state is quite complex. The main input to the nitrogen pool in natural ecosystems is from reduction of atmospheric and dissolved nitrogen to ammonia through the biological process of nitrogen fixation, carried out by diazotrophs using the enzyme nitrogenase. The ammonia produced by nitrogen fixation is usually assimilated into amino acids and hence to protein and other nitrogen-containing compounds. Now, the question is how the entire process is being operated? Well, there occurs an elaborate exchange of signals, the pattern of which shows a classic paradigm of co-evolution. One such symbiosis occurs through nodulation that is found in roots of leguminous plants in association with rhizobia. At molecular level, separate host specific [nodulin/Nod] genes and rhizobial specific [nodulation/nod] genes are involved in nodule formation. Flavonoids are the prominent inducers of rhizobial nod gene expression. The proteins which are essential for the initial identification of the specific Nod factor are a type of pattern recognition receptors. These receptors, present on the plasma membrane of root hairs are transmembrane kinases which have extracellular region bearing LysM domains, a transmembrane domain, and a cytosolic domain. Mutations in any of these domains can cause severe nodulation defects and plant is unable to nodulate. Signaling pathway leads from Nod factor to transcription via calcium spiking recognized by calcium-calmodulin activated protein kinase by the action of CYCLOPS protein which is a DNA-binding transcriptional activator. It induces nodule organogenesis. Both early nodulins and late nodulins play significant role in nodulation. In contrast, the symbiosomes provide microaerobic nodule environment which is suitable for mitrogen fixation by the bacteroids. Although more genes and other factors are required for infection other than just signaling factors, these together constitute to the

signaling pathway or nodulation signal transduction which is the prime topic of discussion in this presentation.

# Introduction

Nodulation is the process in which Nodules, the specialized structures are formed in roots of certain plants, notably legumes and alder when infected by nitrogen-fixing bacteria in which the diazotroph directly provides the host plant with fixed nitrogen in exchange for other nutrients and carbohydrates as part of their symbiotic association.

- Common examples are the Legume-Rhizobia symbiosis and the *Frankia*-Actinorhizal Symbiosis.

FIG: Photo of root nodules on pea (*Pisum sativum*). Source: Long, Stanford University, Palo Alto, CA.

## Associations between host plants and rhizobia

| Plant host | Rhizobial symbiont |
|---|---|
| *Parasponia* (a nonlegume, formerly called *Trema*) | *Bradyrhizobium* spp. |
| Soybean (*Glycine max*) | *Bradyrhizobium japonicum* (slow-growing type); *Sinorhizobium fredii* (fast-growing type) |
| Alfalfa (*Medicago sativa*) | *Sinorhizobium meliloti* |
| *Sesbania* (aquatic) | *Azorhizobium* (forms both root and stem nodules; the stems have adventitious roots) |
| Bean (*Phaseolus*) | *Rhizobium leguminosarum* bv. *phaseoli*; *R. tropicii*; *R. etli* |
| Clover (*Trifolium*) | *Rhizobium leguminosarum* bv. *trifolii* |
| Pea (*Pisum sativum*) | *Rhizobium leguminosarum* bv. *viciae* |
| *Aeschynomene* (aquatic) | Photosynthetic *Bradyrhizobium* clade (photosynthetically active rhizobia that form stem nodules, probably associated with adventitious roots) |

- The most common type of symbiosis occurs between members of the plant family Fabaceae (Leguminosae) and soil bacteria of the genera *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium* (collectively called rhizobia).
- Under conditions of limited nitrogen supply in the soil, there is elaborate exchange of signals between the two symbionts for development of symbiotic relationship.

# ESTABLISHING SYMBIOSIS REQUIRES AN EXCHANGE OF SIGNALS

- Plant genes specific to nodules are called nodulin or Nod genes while rhizobial genes that participate in nodule formation are called nodulation or nod genes.
- The common nod genes-nodA, nodB, and nodC are found in all rhizobial strains but nodP, nodQ, and nodH; or nodF, nodE, and nodL are host-specific.
- Only one of the nod genes, the regulatory nodD is constitutively expressed and it's protein product NodD regulates the transcription of other nod genes.
- The migration of the bacteria toward the roots of the host plant is mediated by a chemotactic response involving certain chemical attractants especially flavonoids and betaines secreted by the roots which activate the rhizobial NodD protein and it induces transcription of other nod genes by binding to their highly conserved nod box at the promoter region.

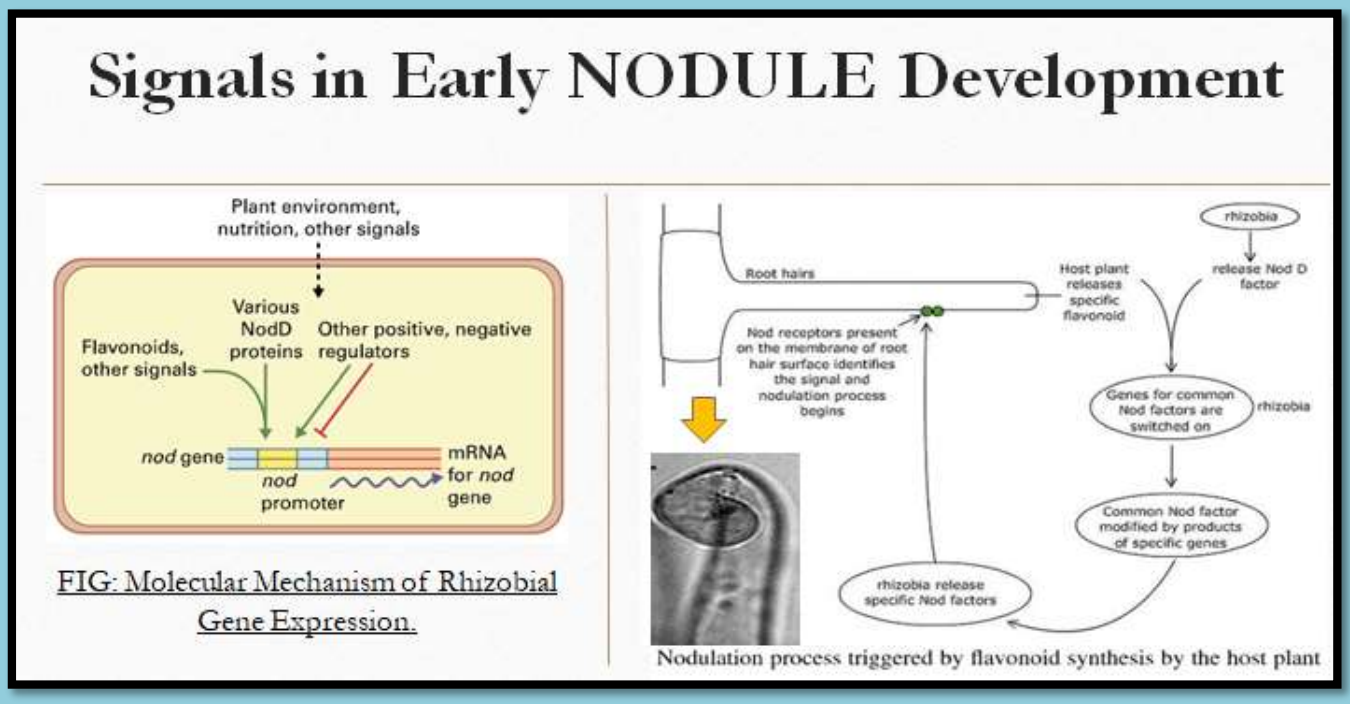


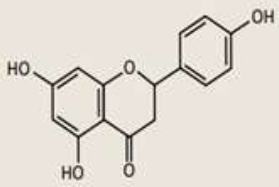

FIG: Molecular Mechanism of Rhizobial Gene Expression.

# What are FLAVONOIDS?

- ✓ Flavonoids, secreted by the host are the prominent inducers of rhizobial nod gene expression.
- ✓ A bacterium's nodD genotype is a primary determinant of the preferred inducer structure.
- ✓ Flavanone is one such example of the most common type of flavonoid inducers which is active on it's specific host *Rhizobium leguminosarum.*

**Host compounds that actively induce *nod* gene expression**

**Flavonoid inducers**

| Category | Name/activity | Specific structure |
|---|---|---|
| Flavanone | Naringenin, a flavanone active on *R. leguminosarum* bv. *viciae* |  |

# Bacteria Synthesize Molecular Signals that Control Plant Development

- What basically these Signals are?
- These are nothing but nodule morphogens or Nod factors encoded by bacterial nod genes which are lipooligosaccharides, fatty acid derivatized oligomers of chitin [β-1,4-linked N-acetylglucosamine (GlcNAc)].
- The core Nod factor structure is a chitin backbone of three to five GlcNAc residues with an N- acyl substitution on the nonreducing end GlcNAc residue. This basic structure is constructed by three proteins common to all Nod factor-producing strains: NodA, NodB, and NodC.
- Other enzymes, which differ among bacterial species, decorate this core structure with various C-6 modifications on the reducing and nonreducing ends, N-methylation or O-carbamoylation of nonreducing end residues, and other O-substitutions on the inner GlcNAc residues.
- Besides these, the invading bacteria may also require some extracellular polysaccharides (EPS) for their recognition by the host plant for nodule formation(e.g.-Succinoglycan in *Sinorhizobium meliloti*).

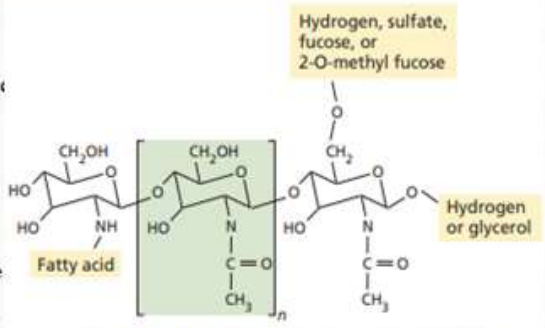



FIG: Nod factors are lipochitooligosaccharides (LCO). The fatty acid chain typically has 16 to 18 carbons. The number of repeated middle sections (n) is usually two or three. (After Stokkermans et al. 1995.)

## PLANT GENES AND PROTEINS REQUIRED FOR NODULATION

The proteins which are essential for the initial identification of the Nod factor are a type of Pattern Recognition Receptors. These include "entry" receptors and "signaling" receptors which are encoded by specific genes.

❑ These receptors have extracellular region bearing LysM domains, a transmembrane domain and a cytosolic domain.

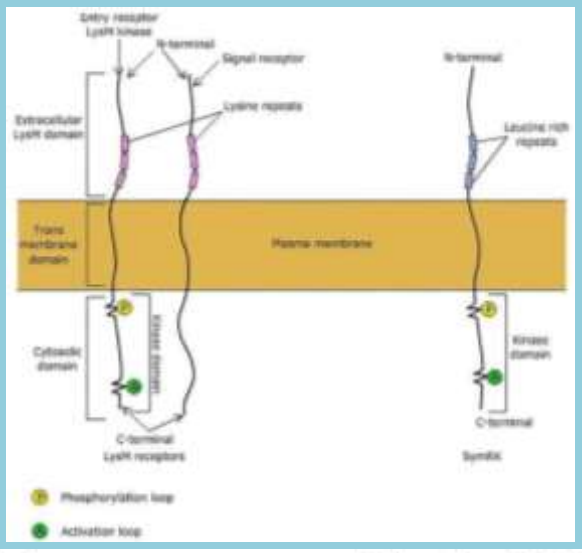
❑ The entry receptor has three extracytoplasmic LysM domains resembling protein elements that bind chitin and chitin-like molecules.

The cytoplasmic domain of the entry receptor includes a kinase domain with several common kinase elements such as the Phosphorylation and Activation loops (shown in picture).

❑ The signaling receptor similarly has three extracytoplasmic LysM domains but lacks the kinase features found in the entry receptor.

❑ The signaling receptor (for example, MtNFP and LjNFR5) is required for any host response to Nod factor. An entry receptor (for example, MtLYK3 and LjNFR1) is required for infection.

❑ The symbiosis receptor kinase, SymRK, which is exemplified by MtDMI2, LjSYMRK, and is required both for symbiosis with rhizobia and establishing symbiosis with broad host range mycorrhizal fungi for nutrient uptake.



Nodulation factor receptors present on root hair surface of the host plant.

## The Central Regulatory Points of Nod Factor Signaling

- Calcium spiking and a calcium-calmodulin-dependent protein kinase are elements of a central regulatory point.
- Ca2+ spiking behaviors must involve at least two components: (1) a channel that releases Ca2+ from a reservoir, and (2) an energy-dependent Ca2+ pump for reuptake of Ca2+ into the reservoir to restore the starting level of ion. The two components typically have a feedback system that sustains or attenuates the behavior.
- In the case of root hairs, the spiking occurs in the nuclear and perinuclear regions and the reservoir is believed to be the nuclear membrane lumen.

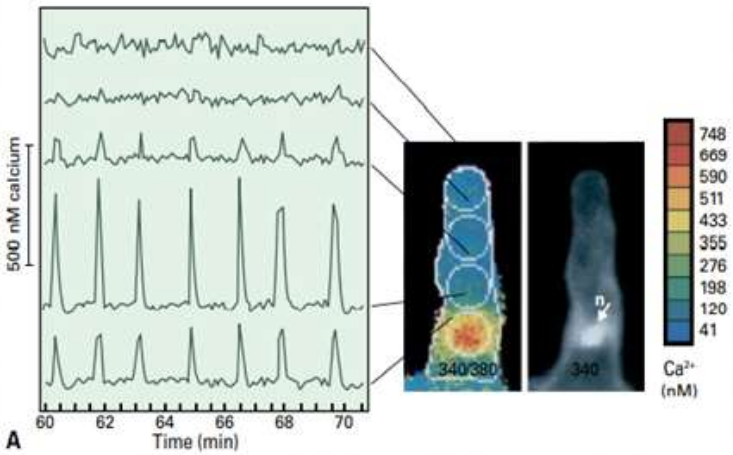



FIG: (A) Calcium spiking in root hairs responding to Rhizobium nodulation factors.

# Activation of Nodulation Genes

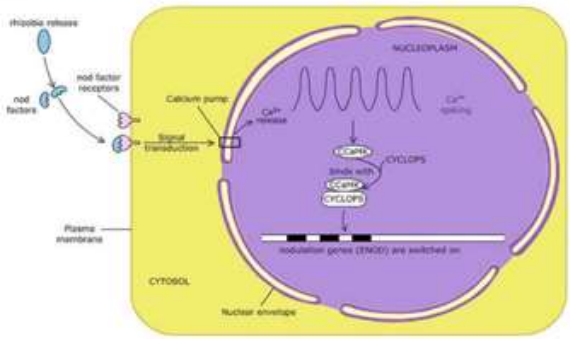



FIG: Calcium spiking is induced in the nucleus of the rhizobia-infected root cells of legume.

- Calcium spiking or oscillation is recognized by calcium-calmodulin-activated protein kinase(CCaMK).
- CYCLOPS, a DNA-binding transcriptional activator, is a direct phosphorylation substrate of CCaMK. CYCLOPS is required for subsequent nodule development.
- CCaMK and CYCLOPS are the key regulators in interpreting Ca2+ spiking in the nucleus and nodule development.
- Proteins, which are expressed in plants early during the infection, are called early nodulins and are encoded by ENOD genes.

# Role of Late Nodulins in NODULATION

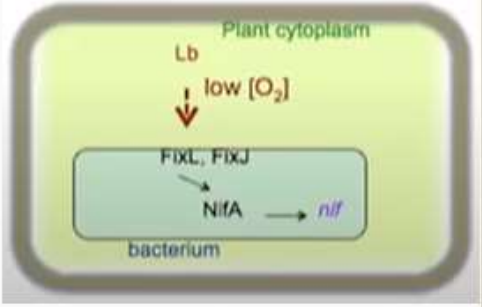



FIG: Mechanism of Action of Leghemoglobin (Late Nodulin).

- Late nodulins are the proteins, which are synthesized later in the symbiotic process. Leghemoglobin is an example of late nodulin which gives pink colour to the nodules.
- Nodules need to be kept under low oxygen concentration in order to prevent nitrogenase enzyme from getting inactivated by oxygen. Leghemoglobin has an active role in delivering O2 to the actively respiring nodules and in regulating the amount of O2 near the site of nitrogen fixation. Leghemoglobin reduces the availability of free oxygen but increases the flux of oxygen to the respiring bacteroids.
- Other late nodulins include proteins required for metabolizing carbon and nitrogen and in facilitating transport of compounds across symbiosome membrane. Some of the nodulins may be involved in transducing signal for cell differentiation in plants and bacteria to accomplish nitrogen fixation.

# Role of Phyto-hormones and some other factors in the NODULE Development

## PHYTOHORMONES:-

- During early stage of the nodule formation, an increase in the cytokinin activity is reported indicating that cytokinin has an important role in the formation of nodule primordia.
- Auxin transport is inhibited in developing nodules. Use of an inhibitor of the auxin transport has, in fact, been found to induce nodule development. However, auxin may be required in the later part of nodule development.
- Ethylene induces calcium spiking or synthesis of early nodulin transcription factors. With ethylene available, host plants require higher amount of Nod factors for the nodulation responses.

## GENES OTHER THAN SIGNALING FACTORS:-

- FLOTILINS: FLOT1 and FLOT2 are both required for normal nodulation in the final stages of symbiosis.
- nif genes and fix genes: Both help in symbiotic nitrogen fixers, fix genes are involved in the development and metabolism of bacteroids and do not have counterparts in free-living forms.
- DNF: Plant genes required for nif function.
  - ✓ DNF1,Signal peptide: Plant peptides control bacterial differentiation.
  - ✓ DNF2,A phospholipase homolog: A role for uninfected cells.

## Steps of Nodulation

1. Release of specific signals by the host plant which generally are flavonoids and secondary metabolites derived from phenylpropanoid pathway.

2. Recognition of these flavonoids by the specific rhizobial spp. followed by synthesis of nodulation factors (Nod) by the bacteria. Synthesis of Nod factors is under the regulation of nodulation (nod) genes of the bacteria.

3. After recognition of Nod factors, various changes in plant-specific proteins (nodulins) are produced within the host plant which trigger physiological responses associated with nodulation process.

   - First step is the curling of the root hair(Shepherd's Crook) causing trapping of the bacteria inside the curl.

4. Bacteria enter the root hair through infection thread which is formed as a result of invagination of the plasma membrane of the root hair cell. Formation of the infection thread is followed by triggering of cell division in the cortical or cambial cells of the root, causing nodule development.

5. Bacteria reach these dividing cells, which later on develop into nodules, through the growth of infection thread and are released while still enclosed by the cell membrane of the host plant. These bacteria are differentiated into bacteroids inside symbiosome. Bacteroids do not divide further and are capable to fix nitrogen.

6. Nodule-specific proteins (nodulins) are synthesized as a result of expression of the nodulin genes of the host plant.

# Conclusion

Analysis made from this topic leads us to the conclusion that nodulation occurs only when the specific rhizobium and its specific host plant come into combination through mutual recognition of biochemical signals. Here, the plant is creating a flavonoid signal that triggers rhizobium which is the symbiont of the host to transcribe its nodulation genes and those encode enzymes that make a Nod factor. So, this mechanism already have some of the hallmarks of the host-specificity. On the other hand, expression of the nif genes is regulated by the availability of nitrogen and oxygen as transcription of nif genes is activated by the product of nifA, which is expressed in the absence of oxygen. During the final stages of symbiosis DNF genes are required specifically for symbiotic nitrogen fixation. Requirement of nodule specific genes like Enods, nodulins and late nodulins were initially explained. Through candidate gene approach we can further conclude the involvement of Flotilins; FLOT2 and FLOT4 are both required for normal nodulation. However, macro and microarray studies have identified many more genes that change expression upon inoculation with rhizobia and we can not rule out any possible changes induced by these genes hence as of now we can just say that signaling mechanisms controlling the entire nodulation process is quite complicated and further research in this field is required to get answers of the undefined questions.

# Acknowledgement

I would like to express my special thanks and gratitude to my teacher Dr. Tripti Roy as well as Dr. Debjani Sinha Roy and all our respected departmental teachers who gave me the opportunity to do this presentation on the topic, Signaling Mechanisms with Respect to Nodulation, which also helped me in doing a lot of Research and I came to know about so many new things. I am really thankful to them. Secondly I would also like to thank my family and friends who helped me a lot in finalizing this presentation within the very limited time frame.

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- Plant Physiol. 2006 Feb; 140(2): 671–680. *doi: 10.1104/pp.105.072132. PMCID:PMC1361333 PMID:16407449* Colby G. Starker,2 Adriana L. Parra-Colmenares, Lucinda Smith, Raka M. Mitra,2 and Sharon R. Long* Department of Biological Sciences, Stanford University, Stanford, California 94305.

# Paper-XXVI

# G-PROTEIN MEDIATED SIGNAL TRANSDUCTION

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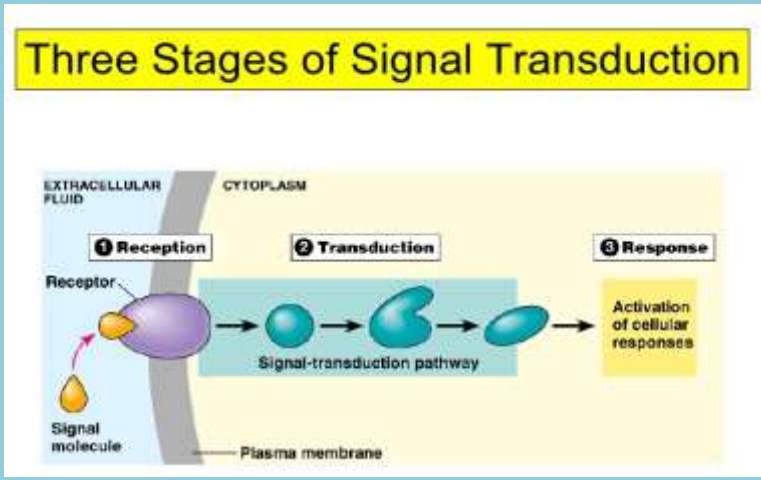
# Abstract

Signal transduction (also known as cell signaling) is the transmission of molecular signals from a cell's exterior to its interior. Signals received by cells must be transmitted effectively into the cell to ensure an appropriate response. This step is initiated by cell-surface receptors.perceive many extracellular signals and transduce them to heterotrimeric G proteins, which further transduce these signals intracellular to appropriate downstream effectors and thereby play an important role in various signaling pathways. GPCRs also regulate cell cycle progression. G-proteins are specialized proteins with the ability to bind the nucleotides guanosine triphosphate (GTP) and guanosine diphosphate (GDP).

# Introduction

Signal transduction ( also known as signalling ) is the transmission of molecular signal from a cell’s exterior to its interior. It is the mechanism by which signals are detected at the cell surface by membrane bound receptors and transmitted into its cell interior, resulting in changes in the cell’s behaviour and gene expression.



# HISTORY

Nobel Prize in Chemistry in 2012 was awarded to Brian Kobilka and Robert Lefkowitz for their work that was "crucial for understanding how G protein-coupled receptors function".There have been at least seven other Nobel Prizes awarded for some aspect of G protein–mediated signaling.


# WHAT IS RECEPTOR ?

•Receptors are proteins that contain a binding site for a specific signalling molecule or ligand.

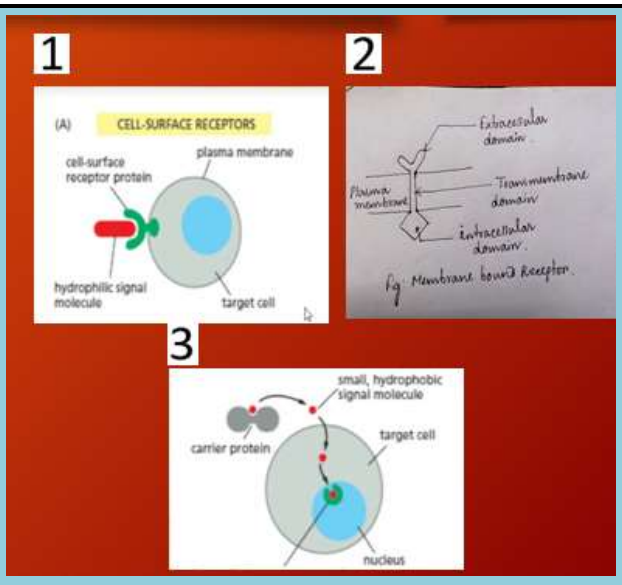
•Receptors are of two types:

1. Plasma membrane bound receptor/membrane bound receptor:

Plasma membrane bound receptors has three domains: Extracellular domain ,Transmembrane domain and intracellular domain.

2. Cytoplasmic receptor:

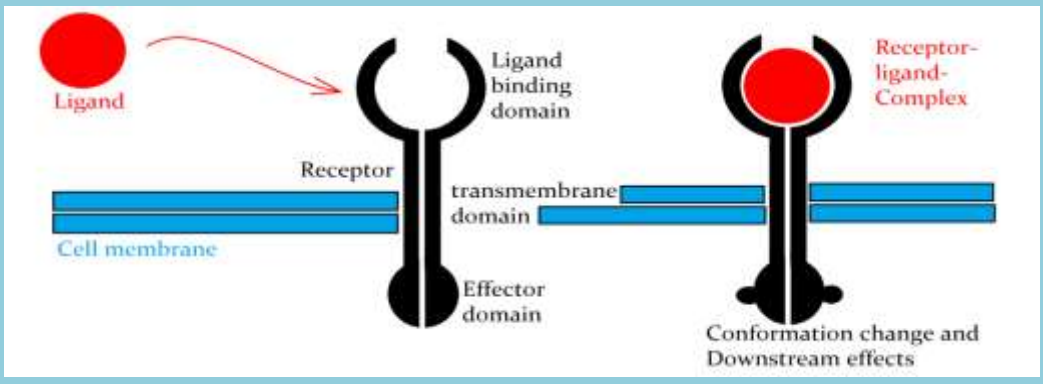
•Cytoplasmic receptors has extracellular and intracellular domain. Transmembrane domain is absent here.




# WHAT IS LIGAND?

•All signals are called ligand. A ligand is a substance that forms a complex with a receptor.

•The name ligand came from the name ligare which means 'to bind'




# WHAT IS PRIMARY MESSENGER?

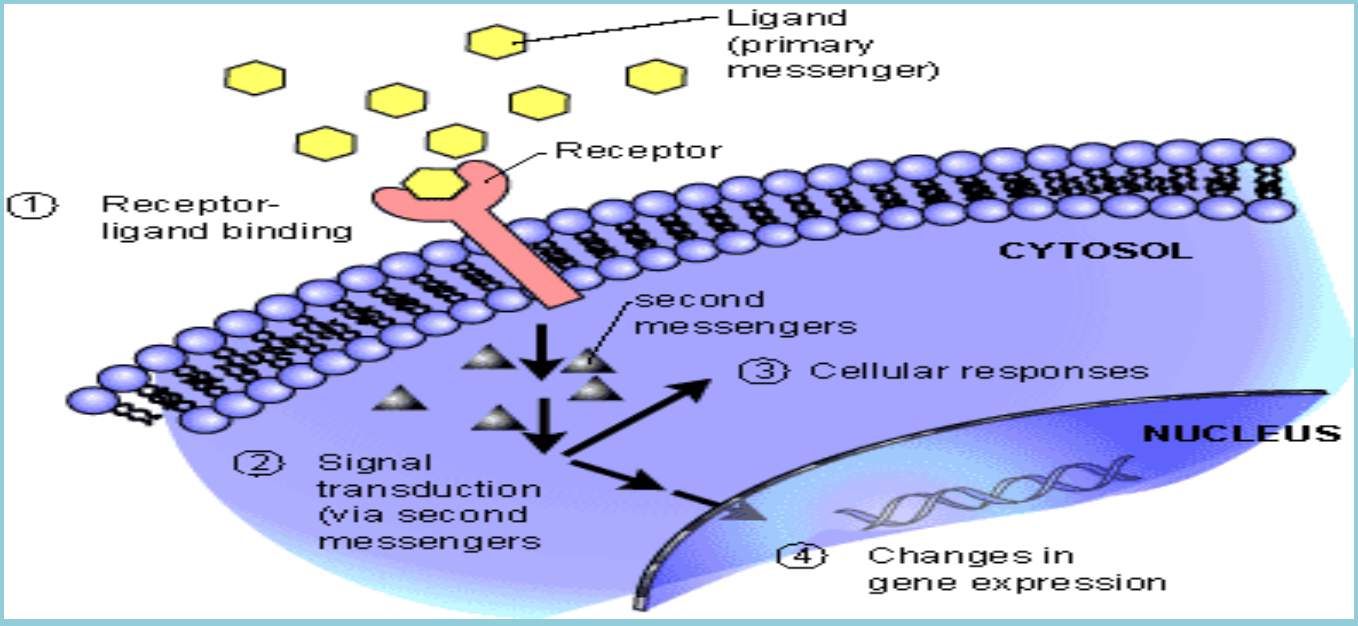
•According to some scientists the signalling molecules or ligands are called primary messengers. According to another group of scientist when ligand bind to receptor the complex is called primary messenger.

•Examples of primary messengers are hormone, steroids etc .

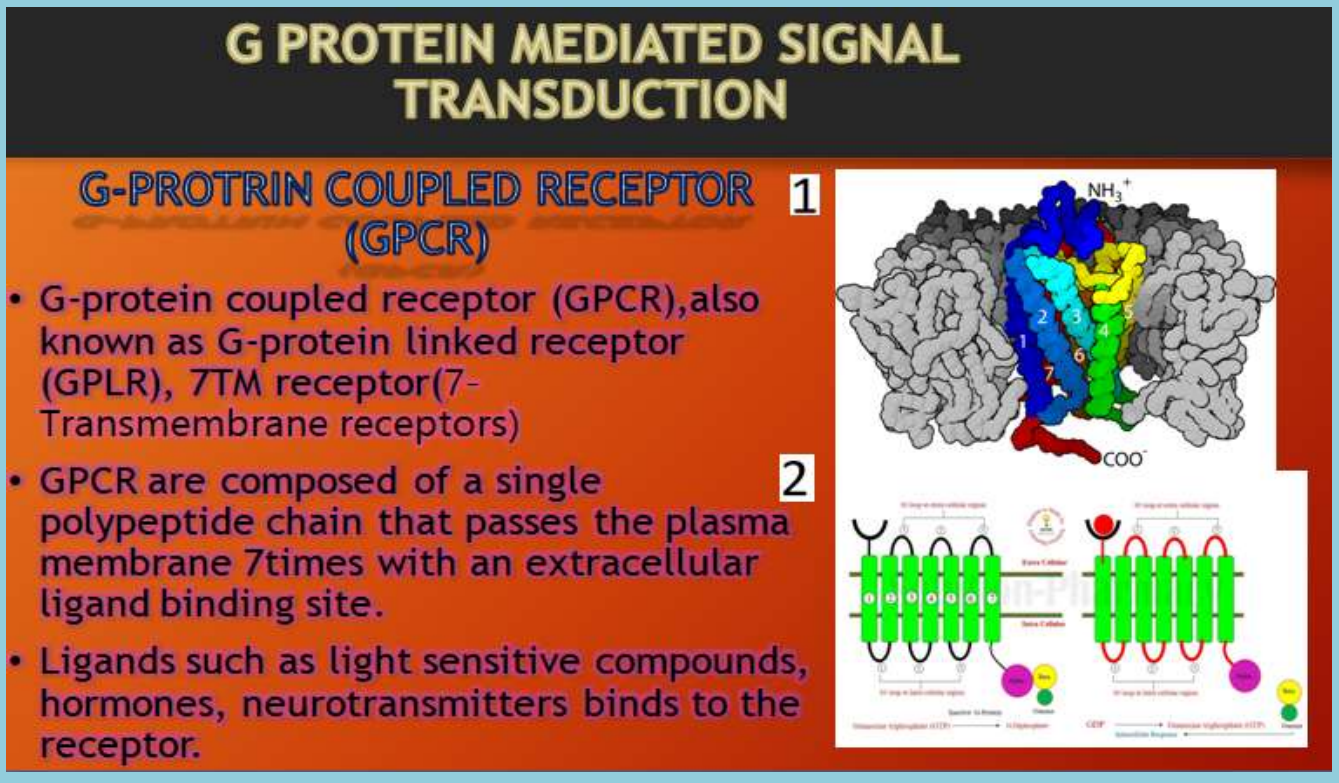
# WHAT IS SECCONDARY MESSENGER?

•Secondary messengers are small intracellular molecules that are activated by extracellular signals and relay the signal to the interior of the cell. Those molecules are activated forming a cascade.

•Examples of secondary messengers are cyclic AMP(cyclic adenosine monophosphate), IP3(Inositol triphosphate or inositol 1,4,5 triphosphate) etc




# G PROTEIN MEDIATED SIGNAL TRANSDUCTION




•G protein-coupled receptors are found only in eukaryotes, including fungi, animals, plants, and protozoa etc.

• The ligands that bind and activate these receptors include light-sensitive compounds, pheromones, hormones, and neurotransmitters and vary in size from small molecules to peptides to large proteins.

# G-PROTEIN

•G-protein (Guanine nucleotide binding protein ) are heteromeric proteins .It involved transmitting signals and as molecular switches.
•It is an integral membrane protein.
•G-protein consists three subunits –Alpha(α),Beta(β),Gamma(γ).

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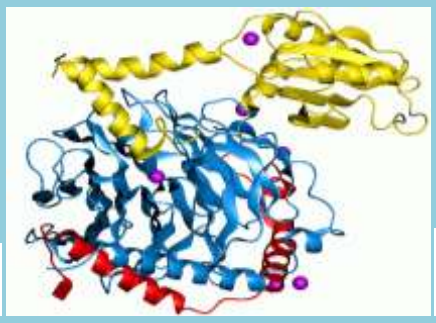

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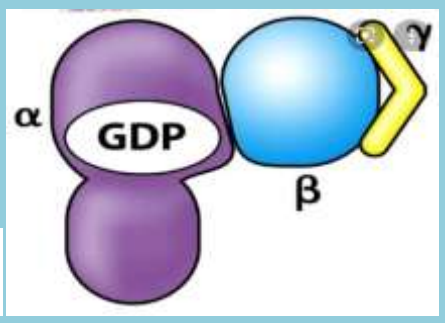
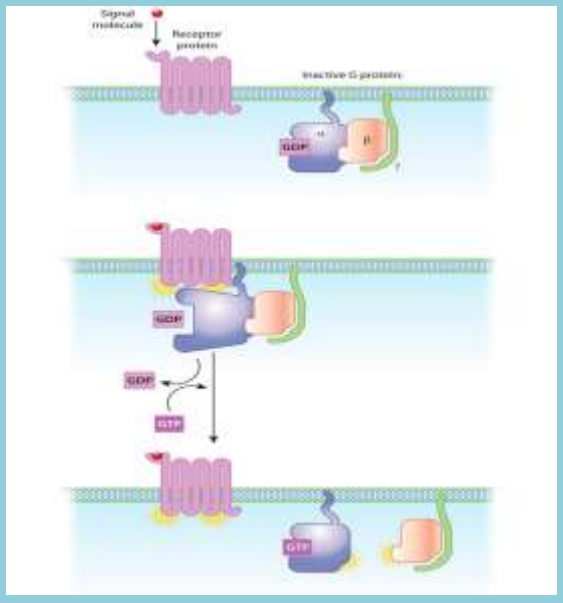
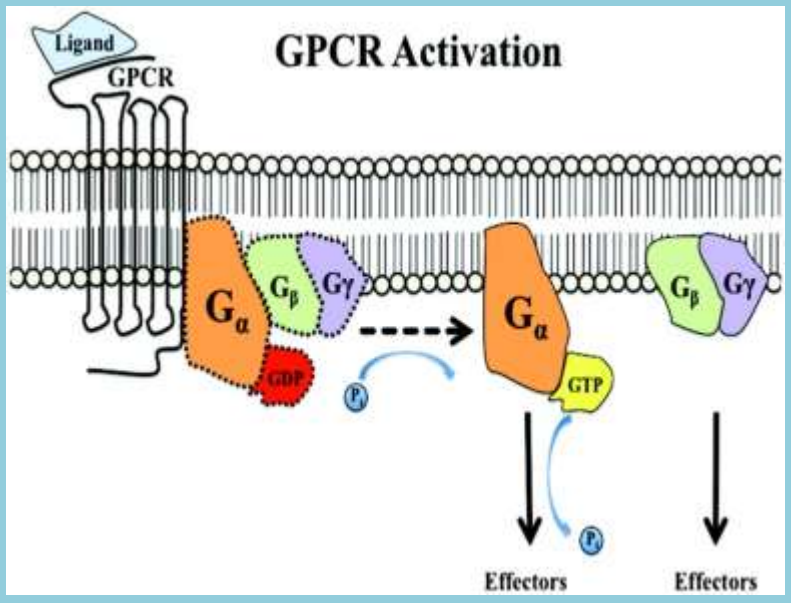



## ACTIVE AND INACTIVE STATE OF G-PROTEIN

•In the inactive state (when there is no ligand bound to receptor ), a GDP molecule binds to alpha subunit of G – protein. When signal molecule binds to receptor the G-protein gets activated and the alpha subunit releases its GDP and binds to GTP molecule.
•Binding of GTP helps in detachment of the G-protein from the receptor resulting in the separation of alpha and beta-gamma complex.





# G-PROTEIN MEDIATED PATHWAYS

Secondary messenger systems involved in signal transduction :

1. cAMP(cyclic adenosine monophosphate) mediated pathway

2 .Phospholipase mediated pathway

## SIGNAL TRANSDUCTION MEDIATED BY cAMP

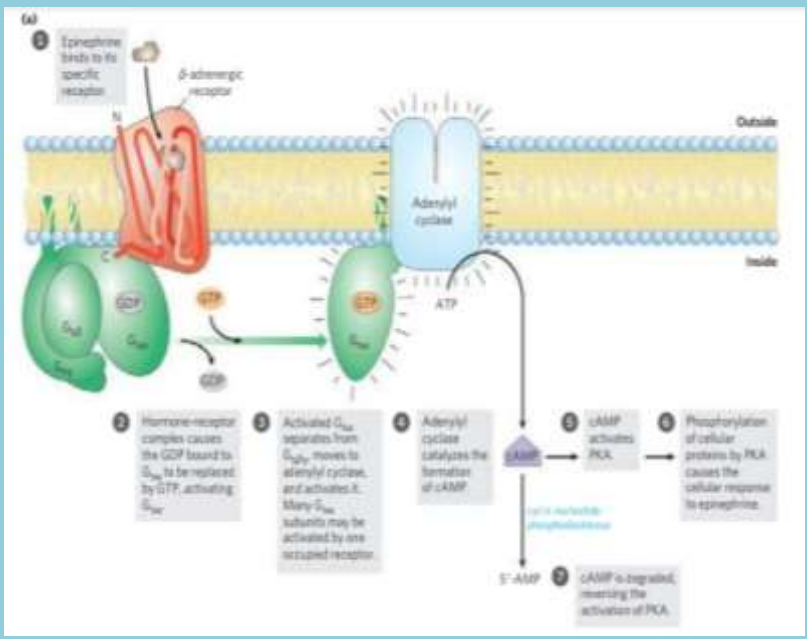
•The cAMP(cyclic adenosine monophosphate) dependent pathway is also known as the adenylate cyclase pathway.

•When GPCR(G-protein coupled receptor) activated by the extracellular ligand a conformational change is induced in the receptor (in the intracellular domain ) that help to attach the intracellular heteromeric G-protein .

•Then the G-protein is activated and then the activated alpha subunit bind to and activate the enzyme adenyl cyclase ,which in turn catalyzes the conversion of ATP into cAMP.

•Increase concentration of the secondary messenger cAMP may lead to the activation of an enzyme called protein kinase A (PKA).

•The activated PKA results in the gene expression.



# PHOSPHOLIPASE MEDIATED PATHWAY

- Activated alpha subunit of G-protein activate the membrane bound enzyme phospholipase-c.
- This enzyme then cleaves the inositol phospholipid into two part ,inositol 1 ,4,5 triphosphate (IP3) and diacylglycerol (DAG).
- IP3 then diffuse in the cytosol and DAG remain embedded in the plasma membrane.
- The IP3 bind to the ca2+ channel of endoplasmic reticulum and vacuole and thus open the ca2+ channel.
- The ca2+ ions are released from the vacuole and ER.
- Then together with ca2+ ion DAG activates protein kinase C (PKC).
- The activated PKC then activate several gene expression.

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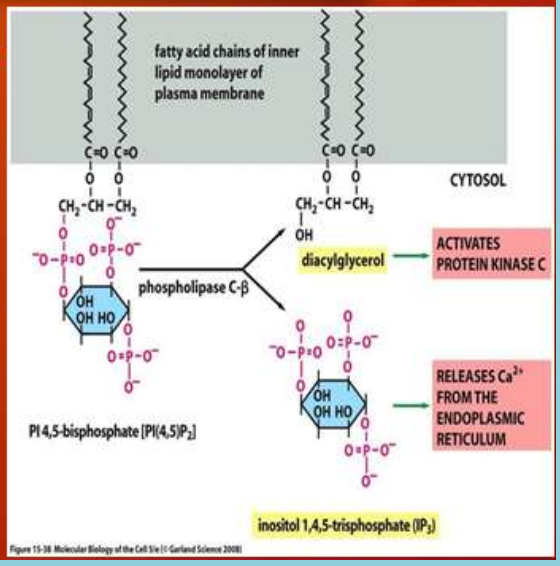


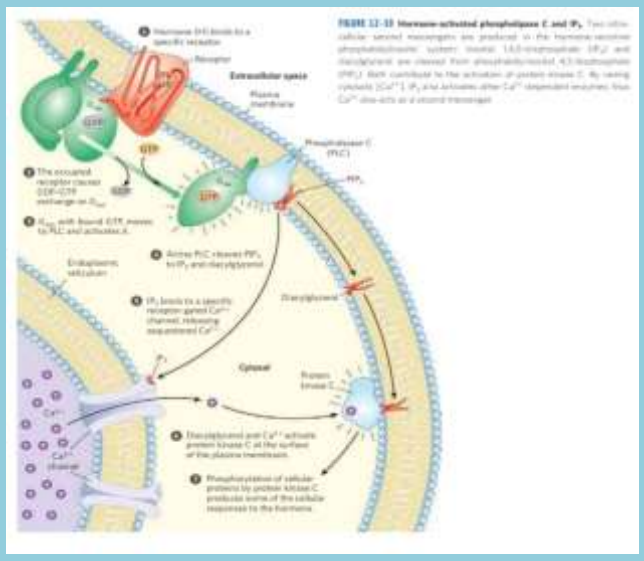

# ROLE OF G-PROTEIN

- Gibberellin action in seed germination.
- Action of metabolic enzyme.
- Embryonic development.
- Immune system activity and inflammation.
- Motility.
- Action of medicine.

# Acknowledgement

I would like to express my gratitude to Dr.Tripti Roy and Dr.Debjani Sinha Roy, Associate Professors of Department of Botany, Bethune college for giving me the opportunity to present this topic. It has been a learning experience for me. Thank you ma'am for your constant support, guidance and mentorship. Then I should mention the name of Dr.Arun Banerjee from whom I Initially received the knowledge regarding this topic.

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# Paper-XXVII

# BIOLOGICAL CLOCK IN PLANTS

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# Abstract

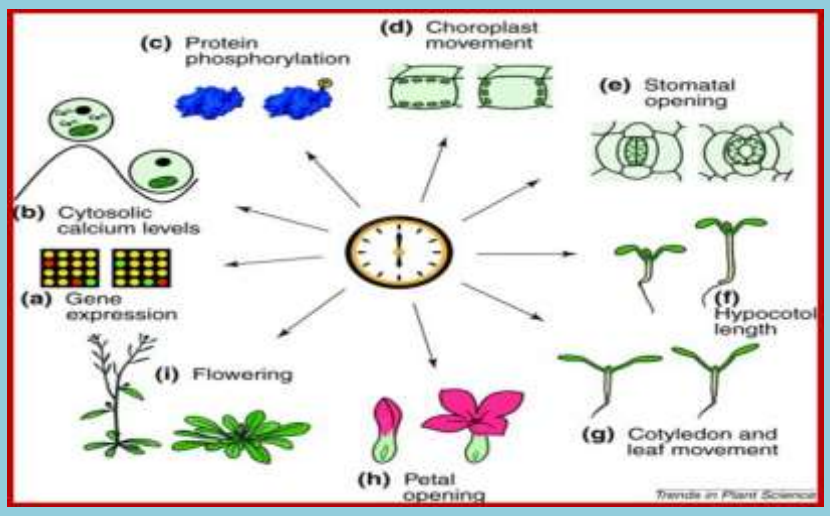
A biological clock is an internal (endogenous) timing system that continues without any external cues and controls the activities of plants and animals. Advantages of a Biological Clock: Enables plants to anticipate environmental changes such as sunrise and maximise photosynthesis.

In this presentation I have tried to explain the characteristics and mechanism of the clock.

# Introduction

Franz Halbery in 1950 coined the term circadian rhythm

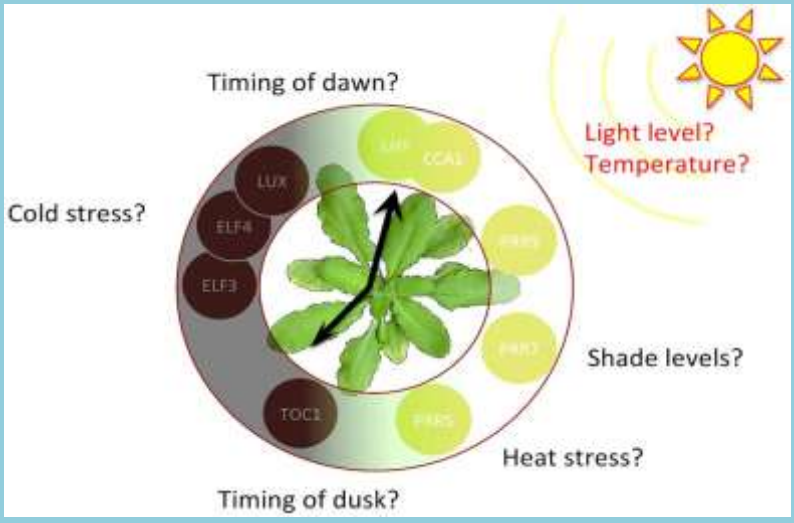
In plants, leaf and patel movements,stomatal opening and closing, growth and sporulation pattern, as well as metabolic processes like photosynthesis and respiration are some examples of cicadian ryhm.



# CIRCADIAN CLOCK?

- A circadian clock, or circadian oscillator, is a biochemical oscillator that cycles with a stable phase and is synchronized with solar time.
- Such a clock's *in vivo* period is necessarily almost exactly 24 hours (the earth's current solar day). In most living things, internally synchronized circadian clocks make it possible for the organism to anticipate daily environmental changes corresponding with the day–night cycle and adjust its biology and behavior accordingly.

**DEFINITION: Plants and Animals are sublected to daily cycles of light and darkness (2 hrs cycle) and therefore often exhibit rythmic behaviour is association with these changes. This behavior is called biological clock or circadian rhythm.**




# CHARACTERISTIC FEATURES

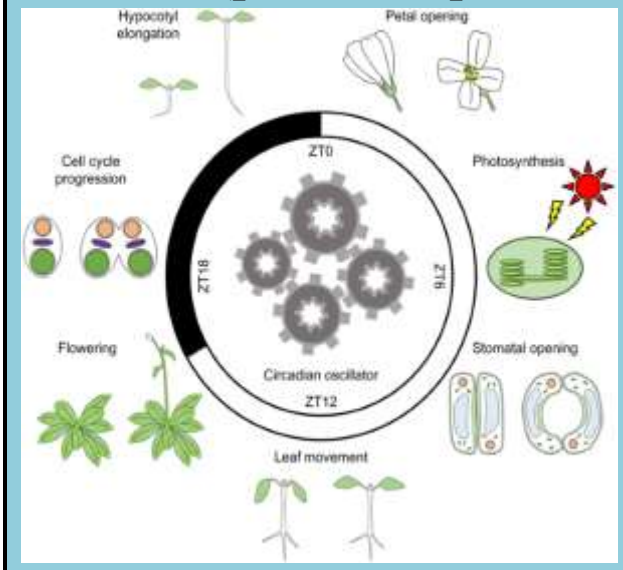
**The cyclic rythms arises from cyclic phenomenon are supposed to follow 3 parameters.**

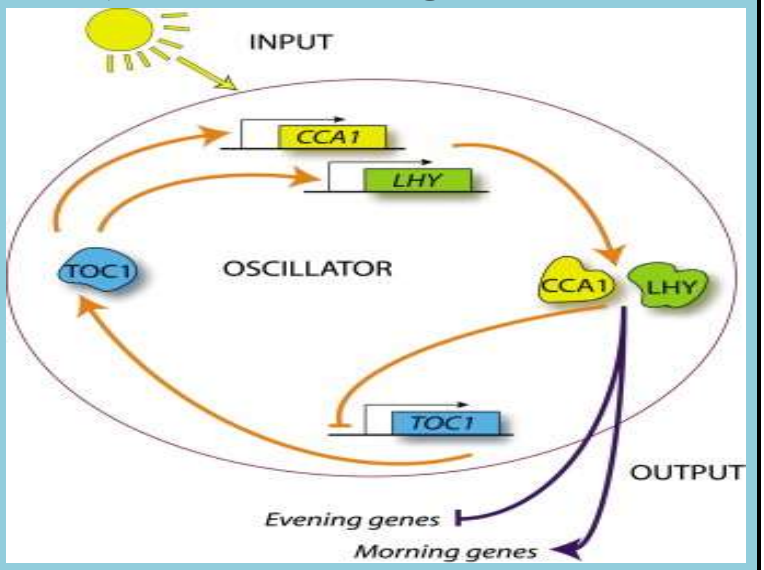
- **Period : The timebetween comparable points in the repeating cycle. Typically the period is measured as the time between consecutive maxima or minima.**

- **Phase: Any point in the cycle that is recognised by its relationship to the rest of the cycle.**
- **Amplitude:usually considered to the distance between peak and trough. The Amplitude or a biological rhythm can vary while period remains unchanged.**

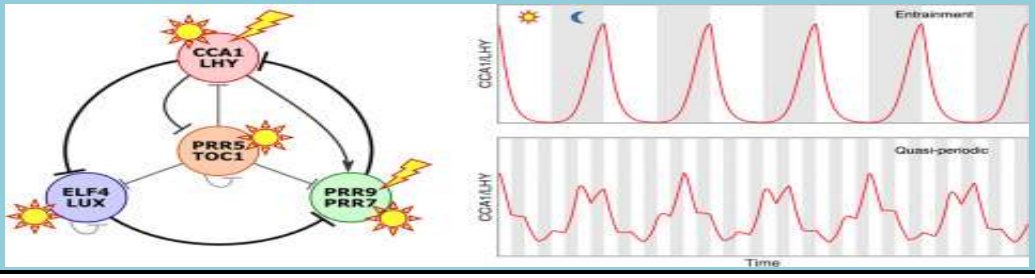
# MECHANISM

**Biological clock in plants is governed by a set of clock genes. In Dabidopsis, CCA1(Circadian clock associated 1),LHY(Late elongated Hypocatyl),TOCI(Timing of CAB expression 1) and PRR(pseudo response regulator) controls Biological clock.**




- **In the morning, LNY and CCA1 are synthesized. This upregulates their inhibitors PRR& and PRR9, and they inhibit LHY and CCA1 by a negative feedback loop.**
- **LHY and CCA1 inhibit expression of evening gene TOCI.**
- **After inhibition of LHY and CCA1, TOC1 increases of dusk.**
- **A hypothetical gene x again deregulates TOC1 and takes back the plant from evening to morning loop.**



# Conclusion

- **ssr1: role in phyB signaling and in regulation of circadian clock(like ELF3 and GI).**
- **Elf3:arrhythmia in light but remains t=rythmic darkness > light input to clock.**
- **Srr1 circadian phenotype both in light and darkness>required for normal oscillator function.**
- **Elf3 interacts with phyB in vitro > interaction between srr1 and phyB?**

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# Bibliography

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