NITOGEN METABOLISM

Role of Notrogen in Plants : Constituent of proteins, nucleic acids ATP, GTP, Chitin, Vitamins, chlorophyll, alkaloids, cytochomes, hormones. Nitrogen is necessary to plants for heridity, reprodcution , growth metabolism and development.

Sources of Nitrogen to plants :

(1) Atmospheric nitrogen :
$N \equiv N$ (Moelecular inert or elemental form) used by Rhzobium (Legumes), BGA, Lichens, Yeast, Pullularia.

- These **converts atm. N_2 into metabolically usefull amononaium ions (NH_4^+)**. This process is called as biological nitrogen fixation.

(2) NO_3^-, NO_2^-, NH_4^+ in soil :
These are major source of nitrogen to plants.

- **Nitrate ions (NO_3^-)** and **cheif form of nitrogen used by majority of plants.)**

Plants grow in acidic soild & found in forest use ammonium ions (NH_4^+) as major N_2 source.
Nitrate ions are cheif source of N_2 for plants but they can not be u sed directly in metabolic pathway in plant cells, as it is highly oxidised from. So **NO_3^- (Nitrate) first converted into NH_4^+ (ammonium ions)** called **nitrate reduction.** So NH_4^+ ions enters in plant metabolism.

(3) Organic nitrogen in soil : as amino acids, protein body.
Due to death & decay of organism. This is not a major source of N_2.

(4) Insect bodies : for some plants (insectivorous plants)

(5) Urea as chemical/artificial fertilizars

Nitrogen (N_2) Cycle :

(1) Biological Nitrogen Fixtion/Diazotrophy $N_2 \rightarrow NH_3$) : Converstion of molecular or elemental notrogen ($N \equiv N$) into inorganic nitrogenous compounds (NH_4^+) through agency of living organisms is called as **biological nitrogen fixtion** or **Diaxotrophy**
Nitroen Fixing organisms (Diazotrophs)

(A) Free living organisms or non-symbiotic organisms/bacteria : Azotobacter, Aerobacter, Beijerinckia, Heliobacerium (Clostridium, Rhodospirillum, Chromatium, chlorobium, Rhodopseudomonas.

- **Cyanobacteria** (Blue = green algae) - **Nostoc Anabaena, Caulotrhix, Cylindriospermum, Tolypothrix, Aulosira** etc.

(B) Symbiotic Nitrogen fixers :

- **Rhizobium leguminosarum (= Bacillus radicicola)** is association with the root noduels of leguminous plants. None of th ese two partners alone can fix at. nitrogen.

(i) Root nodules of non-legume (15-genera) Angiosperms : Casuorine, Myrica, Alnus, Ceanothus, Elaiagnus, Hippophae, Coriaria.
Filamentous, Actinomycetous bacteria **Frankia** live in root noduels of these non-legume plants
Both **Rhizobium** & **Frankia live freely in soil** but **fix nitrogen only when in symbiotic association** with host plant.

(ii) Root nodules of gymnosperms : Cycadaceae, Podocrpus.

(iii) Leaf Nodules (Phyllosphere) : **Psychotria, Dioscoria (Klebsiella),** Rubiaceae **(Mycobacteria)**

(iv) Lichens : Cyanobacteria (BGA) partner

(v) **Non noduel formin assocaitions :** Between **Azolla** (aquatic fern) & Cyanobacteria **Anabaena.**

(vi) **Paspalum-notatum** grass have loose symbiosis (associative symbiosis) with Azotobacter pospali in roots and Azospirillum with wheat, maize sorghum etc.

(2) **Ammonification :** Conversion of dead organic nitrogenous compounds into ammonia. **Bacillus mycoides, B. ramosus.**

(3) **Nitrification : Oxidatioin of ammonia,** produced by ammonification **into nitrate** by **nitrifying bacteria** is called as **nitrification.**

(i) $2NH_3 + 2O_2 \xrightarrow{\text{Nitrosomonas}} 2NO_2^+ + 2H_2O + 2H^+$

Ammonia

(ii) $2NO_2^- + O_2 \xrightarrow{\text{Nitrobacter}} 2NO_3^-$

Nitrate ions

Some fungi like Aspergillus, Penicillium can also carry out this process.

(4) **Denitrification :** Nitrates or nitrites converts back into molecular or atm. nitrogen by **denitrifying bacteria** is denitrification. Ex. **Pseudomonas**

(5) **Nitrate reduction :**

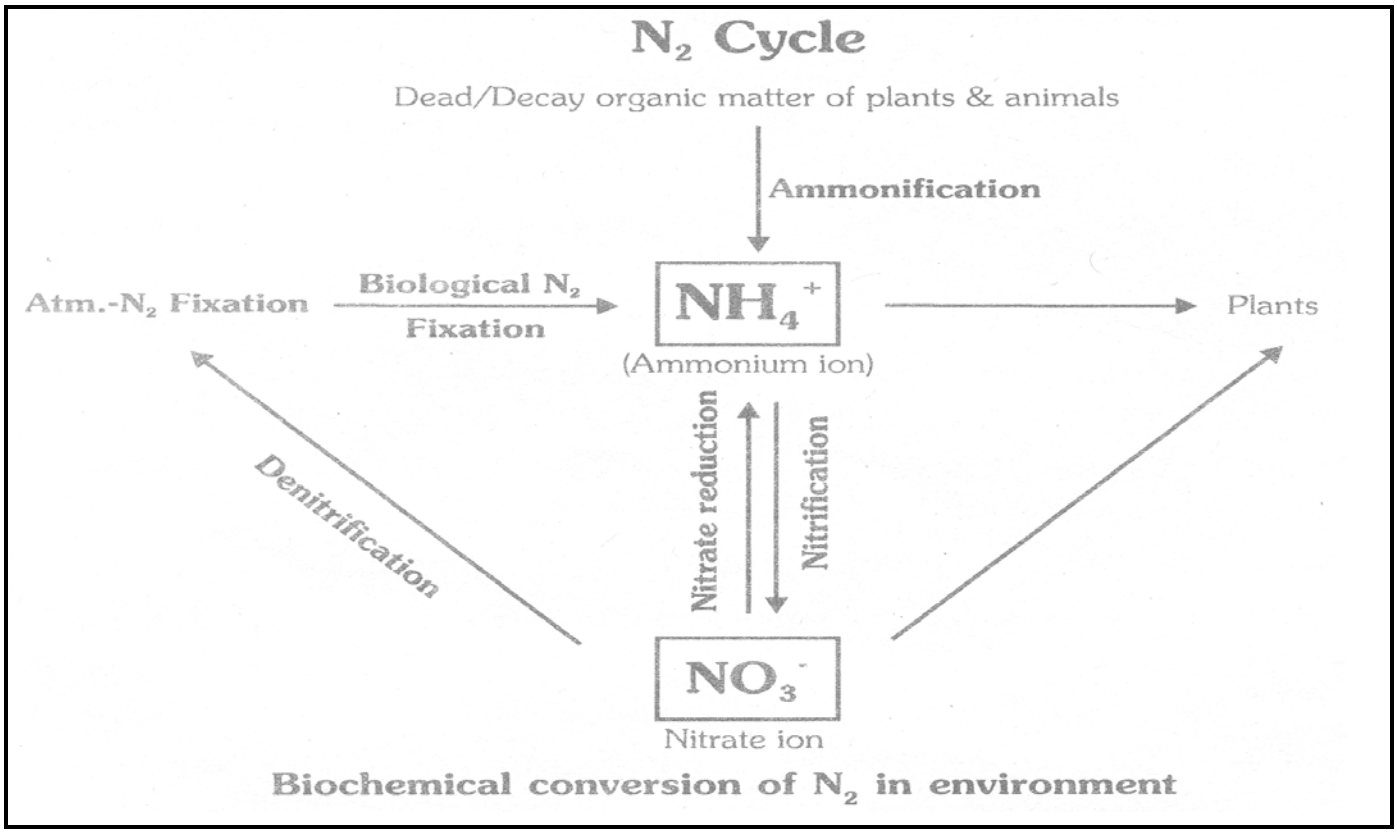
Plants take nitrogen from soil, chiefly in nitrate forms which is highly oxidised form.
so **NO_3 converts in ammonia by following method**

(a) $NO_3^- + NADH + H^+ \xrightarrow{\text{Nitrate reductase}} NO_2 + NAD + H_2O$

(b) $NO_2^- \xrightarrow{2e^-, 2H^+} NOH \xrightarrow{2e^-, 2H^+} NH_2OH \xrightarrow{2e^-, 2H^+} NH_4^+$

or $2NO_2^- + 6H_2O + 4H^+ \xrightarrow{\text{Nitrate reducstate}} 2NH_4^+ + 3O_2 + 4H_2O$

- Nitrate reductate is Molydo flavoprotein isolated by Evans and Naso 1953 from Neurospora and Glycin max leaves.



Biochemical conversion of N_2 in environment

BIOLOGICAL N_2 FIXTION

It is done by symbiotic bacteria & free living bacteria.

Symbiotic N_2 fixation (Diazotrophy) : In **leguminous plants (Fabacea)** by symbiotic bacetriium **Rhizobium.** which form nodules in their roots.

- **N_2** directly **convert into NH_4^+ ion,** which is used in plant metabolism.
- Root noduels act as site for N_2 fixatioin. If contains all necessary biochemical components like enxyme Nitrogenase Leghaemoglobin, required in N_2 fixation.
- Enzyme **nitrogenase** is a **Mo-Fe protein** & catalyse the converstion of atm. N_2 to NH_2.
  It posses two units **unit-Ist is Mo-Fe protein** & **unit-IInd is Ferredoxin** (fe-s protein).
- Nitrpgenanse is extremely sensitive to oxygen. So to protect it form oxygen, nodules contains an **O_2 scavanger** called **leghaemoglobin (Lhb)** and combined with O_2 to form oxyleghaemo-globin (Olhb)
- Leghaemoglobin is **pink** in colour & similar to haemoglobin of vertebrtes. It is synthesised by plant gene (Globin part by plant and heam part given by bacteria).

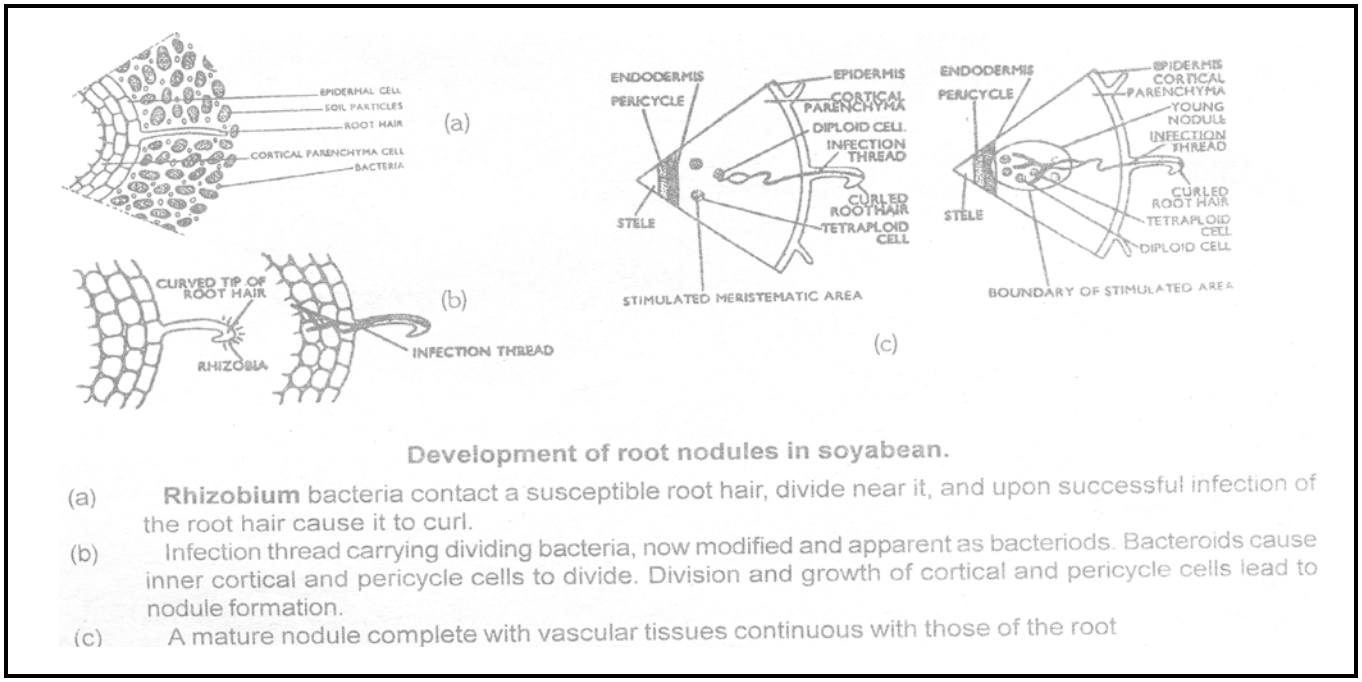
(A) NODULE FORMATION :

It is due to interactiions between bacteria and host root. It occurs in following steps :

(i) **Multiplication & colonization of Rhizobia** at Rhizosphere and attachment to epidermal root hair cells. Initial attraction of Rhizobia to host root is **chemotactic** (Rhicadhesin protein of bacterial cell indentify host root) as **root exude amino acids, sugars, organix acids** and **flavonoids.**

(ii) **Characterstic curling of root hair and invasion of the bacteria** to form an **infection thread,** by the inveginatiion of plasma membrane of root hair cells and it reaches up to the cortex of roots Curling of root hairs is stimulated by **specific complex polysaccharides found on the surfce of rhizobia,** recognised by **Lectins (small protein of host plant root).**

(iii) **Nodule initiation** & **development in root cotex. Mitogenic agents secreted (Kinetin) by bacteria & auxin produced by plant cell promotes cell division & extension** leading to nodule formation. Noduel established direct vascular connection with host for exchagne of nutrients. Root nudule cell have chromosome in double to other somatic cells. Thus nodule cells are polyploid specially **Tetrapoloid**



Development of root nodules in soyabean.

(a) **Rhizobium** bacteria contact a susceptible root hair, divide near it, and upon successful infection of the root hair cause it to curl.

(b) Infection thread carrying dividing bacteria, now modified and apparent as bacteriods. Bacteroids cause inner cortical and pericycle cells to divide. Division and growth of cortical and pericycle cells lead to nodule formation.

(c) A mature nodule complete with vascular tissues continuous with those of the root

(iv) **Release of bacteria form infection thread** and they differentiate as specialized notrogen fixing cell.

- Bacteria continue to multiply during it's path in root hair cells & bacteria distribute in most of cell. The memrance of infection thread bud off to form small vesicles which containing one or more bacteria. Then bacteria stop dividing & enlage & differentiate in **notrogen fixing cells called bacteroid** & it's membrane called **peribacteriod membrane.**

(B) MECHANISM OF BIOLOGICAL N_2 FIXATION :

By Burris. The atm. N_2 is reduce by the addition of hydrogen atoms.

- The three bonds between two nitrogen atoms $N \equiv N$ or dinitrogen are broken & ammonia **(NH_3) is formed by reduction of $N \equiv N$** and the **reduction fo ammonia (NH_3) to form ammonium ions (NH_3)**

N_2 fixation requries 3 components :

(i) **A stron reducing agent - $NADPH_2$/$FADH_2$/$NADH_2$** - from photosynthesis & respiration.

(ii) **ATP** to transfer hydrogen atom to dinitrogen - from respiration & potosynthesis

(iii) **Nitrogenase enzyme.**

- **Step of N_2 fixation :**

(a) Reduction of N_2 to NH_2 :

$$N \equiv N\ 8e^- + 8H^+ + 16\ ATP \longrightarrow 2NH_3 + H_2 + 16\ ADP + 16ip$$

(b) Reduction fo NH_3 to NH_4^+ :

$$2NH_3 + H_2 \longrightarrow \mathbf{2NH_4^+} + 2e^-$$

Over all reaction :

$$\boxed{\mathbf{N_2 + 6e^- + 8H^+ + 16\ ATP \longrightarrow 2NH_4^+ + 16\ ADP + 16ip}}$$

- Biologial N_2 fixation is controlled mainly by four genes :

(i) **NOD gene of host plant :** These encodes no. nodule specific protein called nodulins, synthesis requires for active nodule development.

(ii) **nod, nif** and **fix** gene of bacteria.

SYNTHESIS OF AMINO ACIDS & NITROGEN ASSIMILATION

Nitrogen asimilation - Inorganic NH_3 (Produced by nitrate reduction or biological fixation or obtained form soil as NH_4^+) reacts with a TCA cycle intermediate - α-ketoglutaric aid to form an amino acid **glutamic acid.** This proces known as **Reductive amination or Amino acid Biosynthesis.**

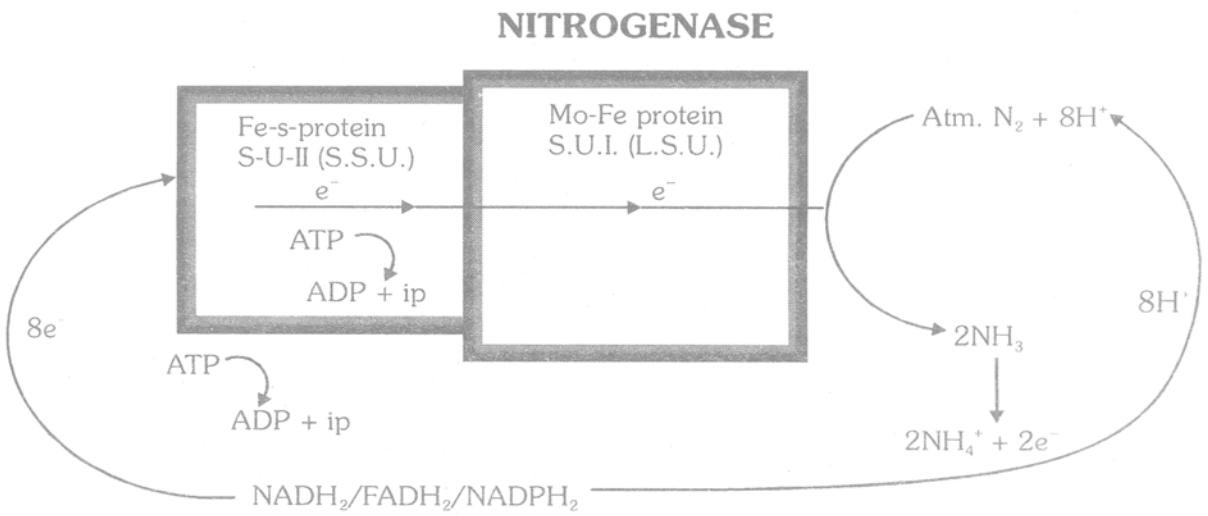
$$\alpha\text{- Ketoglutaric acid} + NH_4^+ + NADPH_2 \xleftrightarrow[\text{dehydrogense}]{\text{Glutamate}} \text{Glutamic acid} + H_2O + NADP$$

Transamination : Transfer of Amino group from glutamic acid to other keto acid is known as transaminatiion. This is a process of formation of other amino acids in plans. **(transaminase enzyme)** Ex.

Glutamic acid + Pyruvic acid $\rightleftarrows$ **Alanine** + α- ketoglutarate

Glutamic acid + OAA $\rightleftarrows$ **Aspartic acid** + α - keto glutaric acid.

- **Glutamic acid is first formed amino acid in plants** & can synthesize different amino acids by transamintion.



Biological N_2 fixation

Mechanism of nitrogen fixation

(a) The 2nd unit (ferredoxin) of nitrogenase, receive electrons from e^- donar ($FADH_2/NADH_2\backslash NADPH_2$) and become reduced.

(b) This reduced 2nd unit is now activated by ATP and form a complex called **ferredoxin ATP complex.**

(c) On other side unit 1st (Fe-Mo protein) of nitrogenase, reacts with molecular nitrogen to form **nitrogenase-nitrogen complex.**

(d) Ferredoxin ATP complex then transfer electron to nitrogenase-nitrogen complex, so that the later gets reduced. This reaction is catalysed by hydrolysis of ATP.

(e) The reduced nitrogenase-nitrogen complex now receives proton (H^+) resulting in formation of ammonium (NH_4^+) ion.

Cataylytic Amidatioin :

Transportaion of fixed N_2/Assimilated N_2 in plants occurs mainly in form of amides especially in leguminous plants as amides are more stable than amino cids and posses high Nitrogen to Carbon ratio (2N to 2C - in Asparagine, 2N to 5C in glutamine (as glutamate posses 1N to 5C)
Formation of amides from amino acids catalysed by enxymes called as catalytic amidation.
In legumes of temperates origine like pea and cloer-Asparagine is translocated in non nodulted plant parts.
In legumes of tropical origin like soyabean and cowpea-ureides are translocated in non nodulated plant parts.

Glutamine synthesis :

Glutamic acid/Glutamate + NH_4^+ + ATP $\xrightarrow{\text{Glumtamine (G.S.) Synthase}}$ Glutamine + ADP + iP

Asparagine synthnesis :

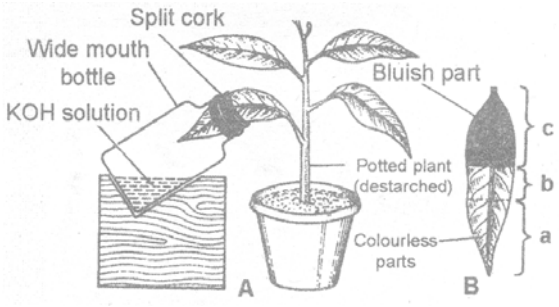
Glutamine/Glutamate+Asprtate + ATP $\xrightarrow{\text{Asparagine(A.S.) Synthase}}$ Asparagine + ADP + iP + Glutamate /α - Ketoglutaric acid

IMPORTANT EXPERIMENTS OF PLANT PHYSIOLOGY

Experiment - 1
CO_2 Necessary for photosynthesis moll half lef experiment

Destarc a potted plant by keeping it in dark for 2-3 days till its leaves do not show positive starch test. Insert one half of a leaf in whide mouthed bottle containing a few c.c. of KOH solution by means of a split cork. Make the connection air tight the leaf half present inside the bottle is unable to get carbon dioxide as the same gets absorbed by potassium hydroxide solution.

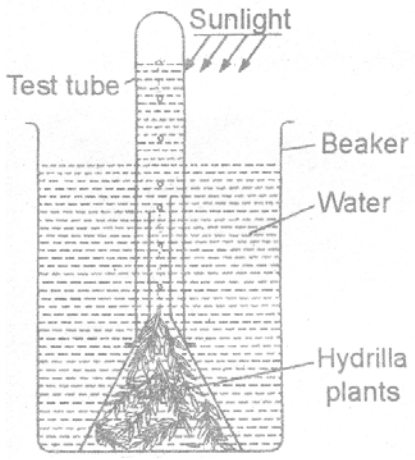
Place the appartus sun light for a few hurs. Remove the leaf under study & tesdt for starch. It will be seen that the inner half of the leaf which was devoid of CO_2 supply does not turn blue, while the oter half shows positive starch test. this experiment shows that photosynthesis does not occurs in the absence of CO_2.



Experiment - 2
Experiment that prove O_2 gas is evolved during photosynthesis.

Place an aquatic plact like Hydrilla at the bottom of alrge waterfilled beaker with the cut end of the stem pointing towards the upperside. Cover the plant by inerting over it a short stememd funnel. P our water into the beaker till it has covered the stem of the funnl completely. Dissolve a bit of sodium bicarbonate in it.

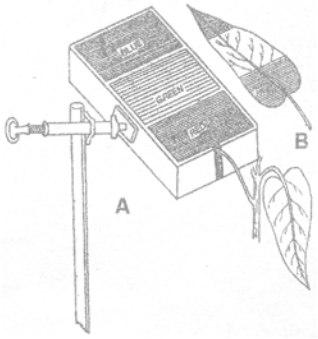
Invert a test tube full of water over the funnel and place the apparatus in bright sunlight. Soon gas bubbles will be seen coming out of the plants and get collected in test tube. When sufficient gas ahs been collected, remove the test tube and intoduce a glowing splinter which will burn brigthly showing that the gas evolved is oxygen.



Experiment - 3
Light is necessary for photosynthesis
(By Ganog's light screen)

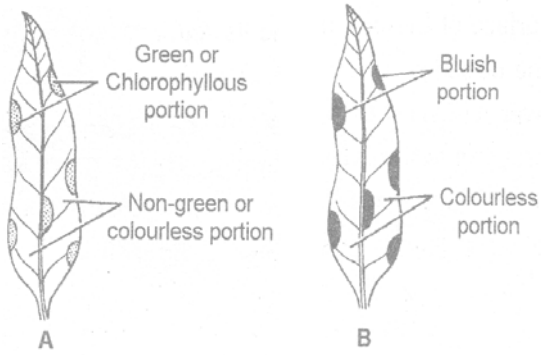
Fir Ganong's light screeen to one of the leaves of a destrached plant.

Place the plant in sulight. The leaf recieve the light only through the stencil. After a few hour pick up the leaf under examination and test for starch with iodine solution the area manufactures starch get blue and thus perform photosynthesis which has recieved light.



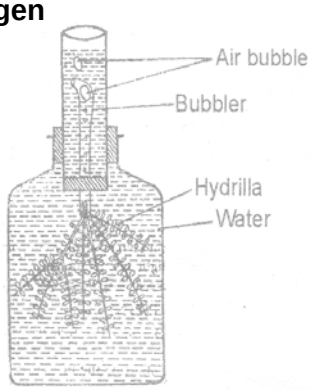
Experiment - 4
Chlorophyll is essential for photosynthesis
(By experiment of varigated leaf)

Pick up a variegated leaf form coleus pothos plant. Which has received light for a few hours. Take a piece of rice paper and place it over the surface of variegated leaf. Mark out the green and non green areas by means of a pencil. 'kill the leaf in bioling wter and remove its chlorophyll by immersing it in warm spirit. Wash and test for starch. Only those parts of the leaf will give positive test which were originally green or with chlorophyll.



Experiment - 5
Measurement of photosynthesis by the rate of evolution of oxygen
(By Wilmott Bubbler)

Take a fesh shoot of Hydrilla plant remove its apical 10cm long portion by giving and oblique cut while keeping it suberged in water. Place it in a test tube 3/4th full of water with the cut end upwards as such or gently tied to a glass rod for suppport. Add some sodium bicarbonate in the water of test tube. Give a fresh oblique cut to the shoot end & place the test tube in sulight. Bubbles of oxygen gas are seen coming out of the cut end of hydrilla plant count the no. of bublles per minute. Rate of reaction increases by adding $NaHCO_3^-$ in H_2O.

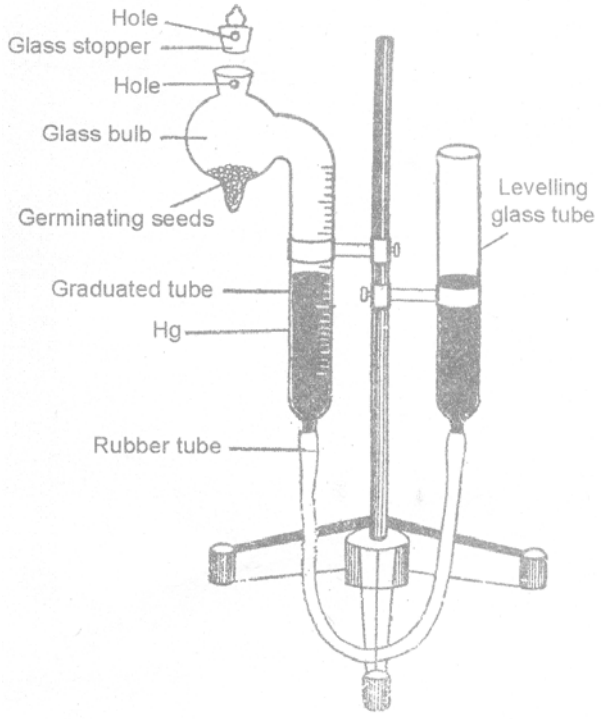


Experiment - 6
Measurement of R.Q. by means of Ganongs respirameter.

Ganong's respirometer has a large stoppered bulb, a graduated side tube and a levellin tube. Both the stopper and neck of the bulb have a hole while can be used to equalise the atmospheric pressure inside the apparatus.

Place the respiring material in the bulb of Ganong's respirometer. Pour saturated common salt solution (CO_2 is sparingly soluble in it) in the levelling tube and not the reading. After an hour note the reading. Calculate the rise (e.g. when fats are respiratory substrate) or fall (e.g. when organic acids are substrate) of salt solution level as +ve, or -ve, cc. Insert-KOH crystal over the salt soltion level in the graduated tube through the levelling tube. KOH shall absorb CO_2. Take a new reading. Let it be V_2CC. V_2CC is the volume of CO_2 librated during respiration. The O_2 used is V_2 plus V_2. R.Q. with therefore be

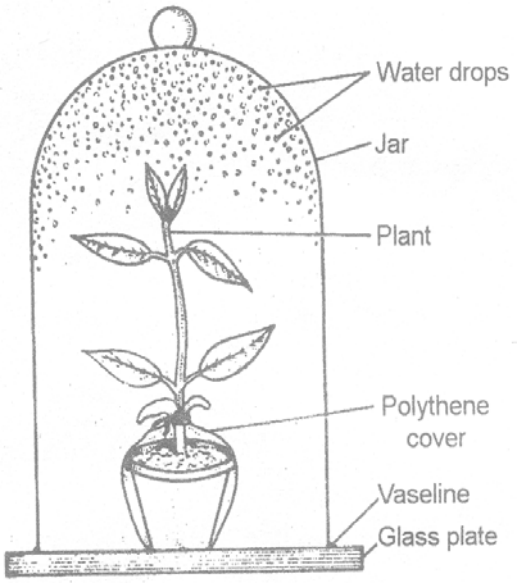
$$R.Q. = \frac{V_2}{V_2 + (-ve \text{ or } +ve\ V_1)}$$



If v_1 is zero, positive or negative, R.Q. will be accordingly unity, less than unity or more than one respectively.

Experiment - 7
Bell jar experiments for demonstration of transpiration

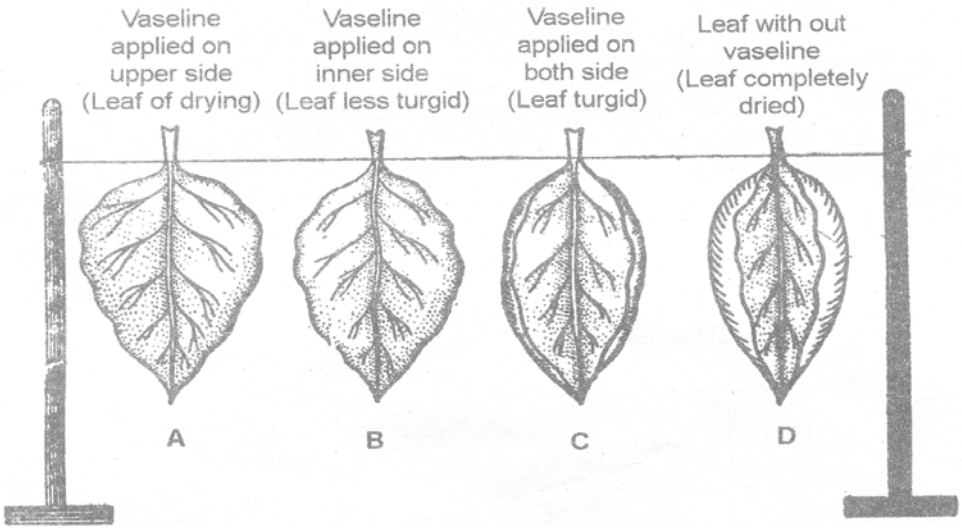
Take a small well watered potted plant. Cover the external surface of the pot and its soil throughly with polythene bag. Place the potted plant on a glass slab in a cool place invert a dry bell-jar over it. Seal the edges of bell-jar with vaseline. After some time drops of water will appear on the inner side of the bell-jar. The water drops have been formed by the condensation of water vapours given out by shoot of the plant in transpiration.



Experiment - 8
Compare the loss of water form the two sides of a leaf
By four leaf experiment

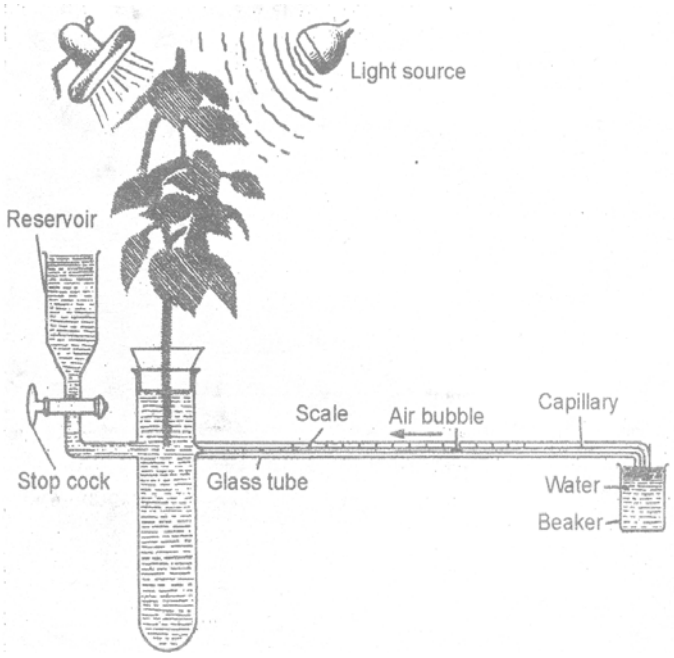
Remove four leaves of Pipal. They should be almost equal size. Seal the cut end of petioles with vaseline. Tie a thread to the petiole of each in the fashion of a loop. Apply vaseline to the upper surface of A, lower surface B and both the surfaces of C. Keep the leaf D as such to function as control.

Weigh the leaves one by one. For weighing each leaf is picked up through the thread attached to its petiote. After initial weighing keeping the leaves hanging from stads by means of their threads for a few hours. Examine them closely. Leaves A & D wil while B and C remain trugid. Weight them again. It will be seen that max loos of weight occurs in D followed by A. Loss of weight is negligible in B. It is nill in case of C. In D leaf both surfaces loss water, while in A only the lower surface is exposed to transpire. The negligible loss of water in B shows that upper surface transpires little.



Experiment - 9
Measurement of Transpiration
By Ganong's potemeter

Fill Ganong's Patometer completely with water. Close the hole of the capillary tube with your finger. Insert a fresh leafy shoot, cut under water, into the mouth of the wide tube by means of cork. Make the connections are tight by applying melted was or vaseline to the joint. Place the apparatus in dyry place or sunlight. Remove your finger from teh free end. An air bubble will enter the graduated capillary tube dip the free end in a beaker containing water. The air bubble will be seen moving forwards.



Note the time taken for the air bubble to travel from one end to other end in tube. As soon as the air bubble reaches the ohter end open the stop cock of the reservior till it is pushed back to the beginning of the horizontal tube. Reapeat the experiment. Note the amount of water absorption in different conditions.

$V = \pi r^2 \ell$

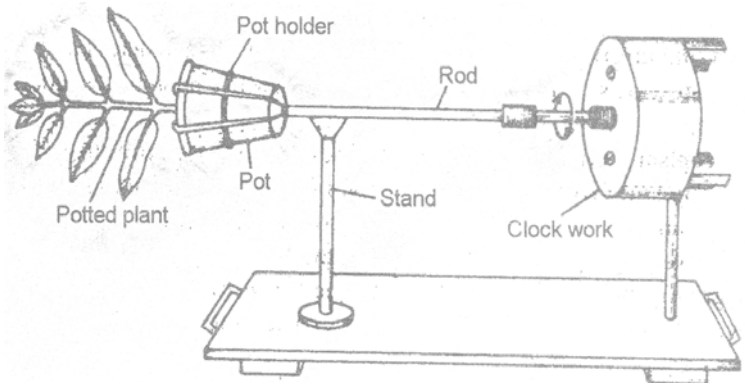
V = volume of water which absorbed/transpired water

π = 3.14

ℓ = distance of movment by air bubble

Experiment - 10
Demonstration of Geotropism by Clinostat

Geotropic curvature results is due to unilateral gravity stimulus can be demostrated by using a Clinostat. Clinostat consist of a disc attached to the tip of an horizontal rod, which can be rotated with the help of a clockwork.

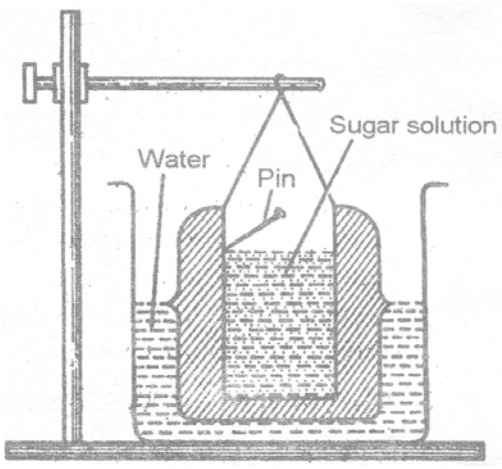


Fix a small young potted plant on a clinostat in horizontal position and rotated, neither the root will bend down nor the stem will curve upward (Nullify the geotropism). If is because in such case the effect of grativity will be uniform all.

Round the stem and root. If however, the plnat lies in horizontal position & not rotated, the roots will receive gravity stimulus only on their lower sides or the effect of gravity will be unilateral. Unilateral stimulus of gravitiy causes unequal distribution of auxin in root tip.(more auxin on upper side than on lowr side). This in turn result in more growth on the upperside and less growth on lowerside and ultimately a +ve geotropism is observed However in case of stem the more conc. of Auxin on lower side promotes mroe growth on that side so stem is negative geotropic.

(Recent findings have impliated ABA in geotropism)

Experiment -11
Demonstration of osmosis by Potato Osmoscope



Take a large sized potato tuber. Cut one side flat. Bore cavity from the other side in such a way that only a thin base is left intact on the flat side. Also remove the skin near the edges of the flat end because the skin of the tuber is impermeable to water. Now place the tuber on its flat cut end in a petri dish half full of water. Fill half of the cavity of the potato tuber by 10-20% sugar solution. Mark the level of sugar solution in the cavity with the help of pin. After some time the level of suger solution reses in the cavity.

The rise in the level of sugar soltuin in the cavity of the pottao tuber indicate that is have obsorbed water number of cells.

The entry of water into sugar soltuin proves that (i) Sugar solution is an oxmotically active solution. (ii) the cytoplasm of the cells of the tuber that lie between the sugar solution and the water of the petri dish act as a single semi-permeable membrne (iii) Water enters the suger solution when it is separated from it by a semi - permeable membrane, the phenomenon being known as osmosis.