Course- B.Sc. (Hons.) III

E-content – Online class 10/9/20

Nitrogen Metabolism

Symbiotic nitrogen fixation –

When nitrogen is fixed by symbiotic bacteria living in association with plant, it is called symbiotic N_2 fixation. This is most common in some leguminous plants, where nitrogen fixing bacteria are present in root nodules. Many species of *Rhizobium* are present in roots of different leguminous plants, such as.

Rhizobium leguminosarum—in Pea

R.meliloti -- in Alfa alfa

R. trifoli ---- in Clover

R. phaseoli—in Beans

R. lupini—in Lupins

R. japonicum – in Soybeans

There are some more symbiotic association of different nitrogen fixing bacteria with non legumes.There are non nodule forming association association in some plants also.Thiese can be enlisted as below-

Casuarina equisetifolia in association with *Frankia* (an acinomycete)

Alnus (Alder) with *Frankia*

Non–nodule forming association-

Lichens-in association with fungi and blue green algae (Cyanobacteria)

Anthoceros (a bryophyte) associated with *Nostoc*

Azolla- (a fern) in association with *Anabaena azollae*

Cycas (a gymnosperm)- Coalloid roots in association with Anabaena and Nostoc

Gunnera macrophylla (an angiosperm) roots in association with *Nostoc*

Paspalum notatum (an angiosperm)- roots in association with *Azotobacter paspali*

Nodule formation-

Rhizobium is a free living soil bacteria which grows near the roots of higher plants but fails to fix nitrogen in this condition. The *Rhizobium* enters into the legume roots and forms a nodule, known as root nodule. A critical specificity is involved in the association of various species of root nodule bacteria and leguminous plants. This step is controlled by specific protein found in host cell called "***lectin***". This acts as determinant of recognition or specificity. ***Lectins*** are the key proteins involved in the legume-*Rhizobium* symbiotic association. Nodule formation is initiated by the infection of root hair root hair by free living Rhizobia. Since these bacteria cannot digest cellulose, they enter the root hair from the tip region where cellulose is absent. Leguminous plants release tryptophan in the soil which is absorbed by *Rhizobium* and is metabolized to produce IAA. *Rhizobia* produce another substance called *root hair curling factor* that causes curling and deformation of root hairs. The root hairs get penetrated by large number of bacteria which collectively convert into an infection thread lined by cellulose. After entering into the root cortical cells, bacteria multiply fast. The Rhizobia are liberated enclosed by a membrane.The cells of bacteria enlarge, assume pleomorphic shape and are called ***bacteroids.*** These bacteroids stimulate polyploidy cells to undergo repeated divisions. As a result of multiple divisions cortical cells develop nodules, which are marked as tumer like nodular structures on the roots. The nodule contains a pink coloured substance known as "*leghaemoglobin*". This substance has a protective role for the enzyme *nitrogenase*, by preventing oxygen to be in contact with nitrogenase. Oxygen inactivates *nitrogenase*.

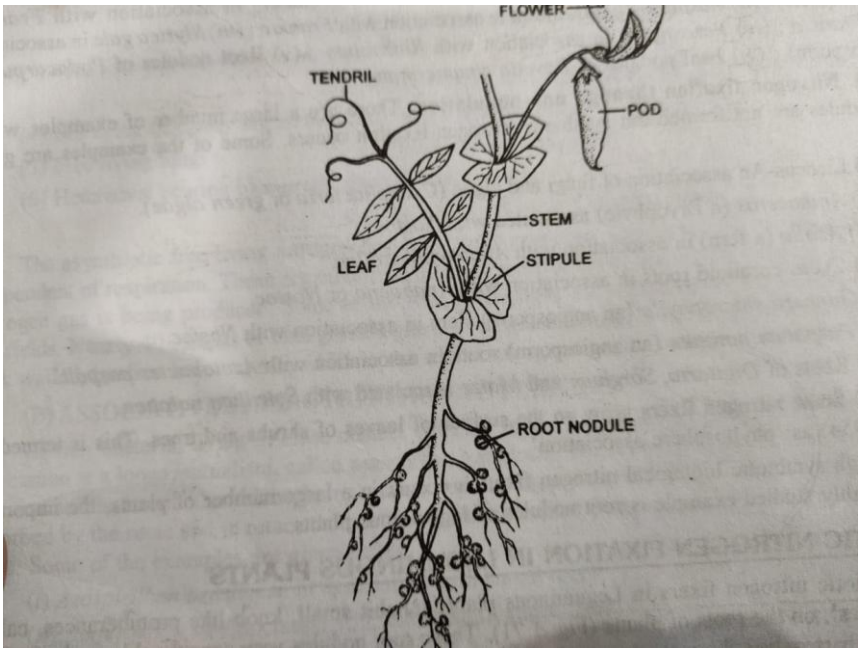


Fig. Root nodules in pea plant

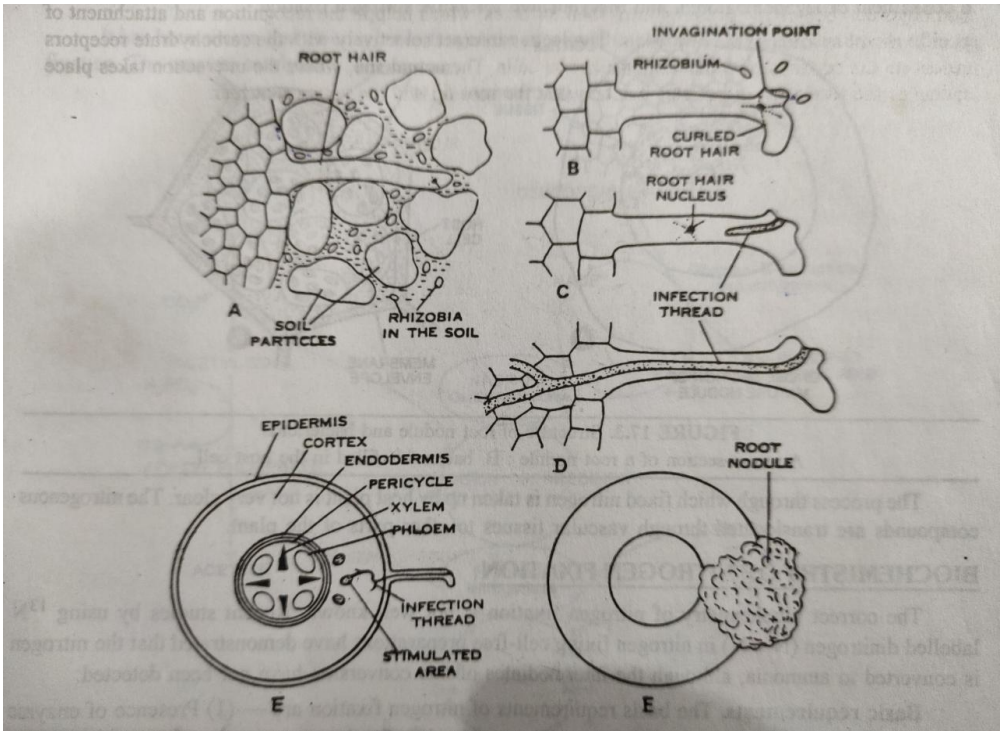


Fig. Showing steps of entry of *Rhizobium* into the root hairs and migration to cortex through infection thread.(Courtesy: Plant physiology, H.N.Srivastava,Pradeep publ. Jalandhar)

Nitrogenase is an enzyme, which reduces N_2 to NH_3 by adding hydrogen. It is made up of two protein sub units, a MO-Fe protein and a Fe-protein. In addition to nitrogen, it reduces another compounds also such as N_2O, acetylene (C_2H_2).It is extremely sensitive to oxygen.

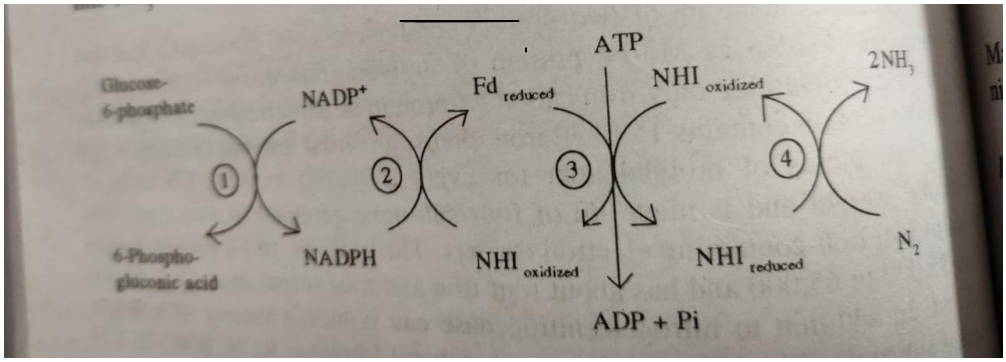
Mechanism of N_2 fixation -

In vitro studies revealed that at least four molecules of ATP are hydrolyzed for each pair of electrons transferred to nitrogen. Thus the reduction of one molecule of nitrogen into two molecules of ammonia requires twelve molecules of ATP because six electrons are required per molecule of nitrogen reduced.

$N_2 + 6H^+ + 6e^- + 12\ ATP \text{ ----------} \rightarrow 2NH_3 + 12ADP + 12Pi$

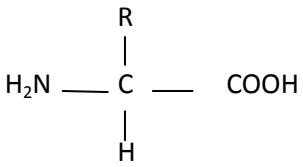
The reduction of nitrogen to ammonia depend on the availability of ATP and reduced substrate which can donate H atoms to nitrogen. ATP is made available from the respiratory process of the bacteroid cells reduced substrate is obtained from host cells. Glucose-6-phosphate is considered as the reduced substrate for thie process and NADPH with ferredoxin function as electron carriers.

The summary of reaction steps can be shown as below-



Amino acid Synthesis

The general formula of an amino acid can be depicted as following :-



Here, R- may be simply a hydrogen group or a complex organic group

H- hydrogen atom

H_2N - amino group

COOH- carboxyl group

Due to presence of one acid or carboylic group (COOH) and one or more basic or amino (-NH_2) group ,amino acid can behave as acids towards bases and as bases towards acids. They are,thus amphoteric compounds. Amino acids are building blocks of proteins. They are one of the important biomolecules which play prime role in cell composition. Once nitrogen is fixed into ammonia, nitrogen enters into the cellular metabolism through amino acids. Amino acid synthesis is directly related with the respiratory metabolism.

There are two pathways of amino acid synthesis-

(i) Reductive amination

(ii) Transamination

Reductive amination

In reductive amination, organic acids,specially α-ketoglutaric acid is aminated by its direct reaction with ammonia. Though, direct amination of oxaloacetic acid, fumaric acid and Pyruvic acid also produce aspartic acid and alanine respectively. Reductive amination is chiefly concerned with the

amination of α- Ketoglutaric acid. α- Ketoglutaric acid is an intermediate organic acid of respiratory metabolism (Krebs' cycle). This is a key pathway of amino acid synthesis. NH_3 is produced as a result of nitrogen fixation, which combines with α Ketoglutaric acid. In the reaction initially an intermediate compound α iminoglutaric acid which in turn is reduced to produce Glutamic acid. The reaction can be shown as following :-

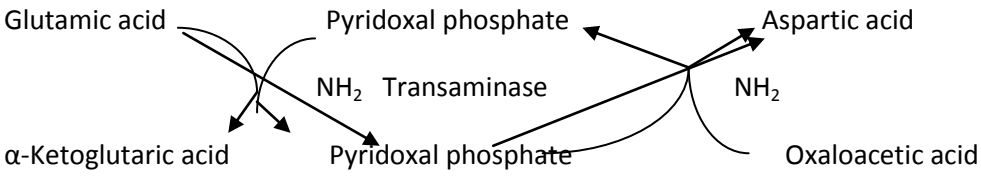
α-Ketoglutaric acid + NH_3 ←====→ α-iminoglutaric acid

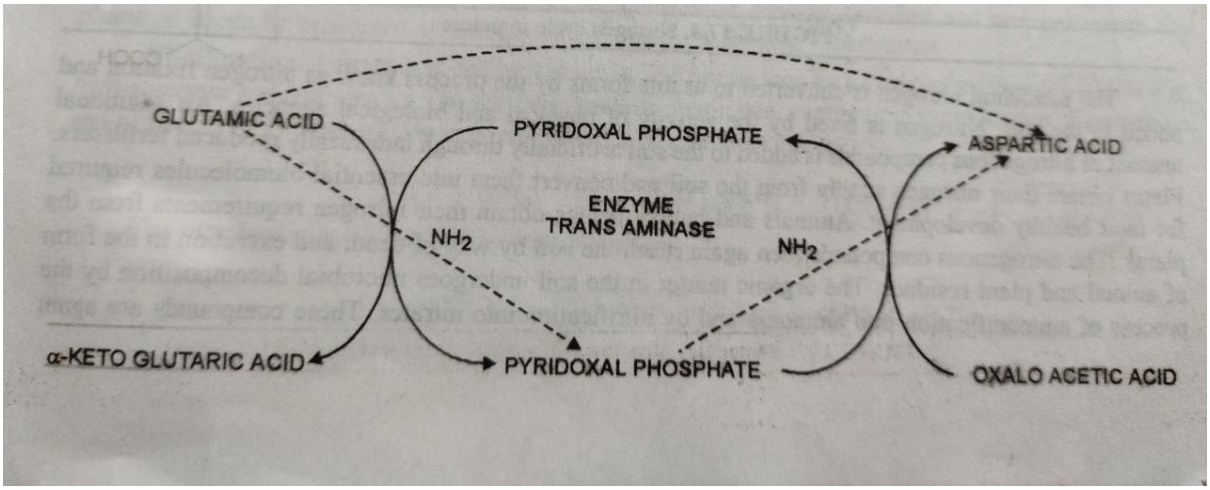
α-iminoglutaric acid + NADH ←====→ Glutamic acid + NAD^+

Transamination- Transamination involves transfer of amino group from one amino acid to an α keto acid by the enzyme transaminase, as a result of transamination, amino acid after losing amino group gets converted to its respective keto acid and α-keto acid which receives amino group is transformed to its respective amino acid.The enzyme that catalyzes Transamination, which are called amino-transferases or transaminases,require the coenzyme pyridoxal-5'-phosphate (PLP).

This reaction can be illustrated as below-

Glutamic acid + Oxaloacetic acid ←=====Transaminase===→ α-Ketoglutaric acid + Aspartic acid





Similarly, Pyruvic acid is converted to alanine by transamination from glutamic acid

Glutamic acid + Pyruvic acid---------Transaminase------→ α-ketoglutaric acid + Alanine

Altogether 17 amino acids are synthesized by transamination from Glutamic acid.