Chapter 07
S.Y.B.Sc Sem. III. BO-202 (Major)
NITROGEN FIXATION

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Introduction

- Nitrogen is one of the important mineral elements found in plants, involved in the synthesis of essential amino acids and proteins
- Plants can only absorb nitrogen from the soil which is in the form of nitrates, nitrites & ammonium salts.
- Nitrogen is associated with many cell components such as nucleic acid, purines & pyrimidine's, porphyrin structure of chlorophyll pigments & cytochromes.
- A variety of prokaryotic organisms have the ability to reduce the atmospheric N_2

BIOLOGICAL NITROGEN FIXATION

- **Definition:** Few plants (Legumes & Non legumes), microorganisms like bacteria, fungi & BGA fix atmospheric nitrogen to its useful forms with special mechanism existing in them which is known as Biological Nitrogen Fixation
- **Nitrification & Ammonification**: Free nitrogen is converted

into NO_2 (Nitrites), NO_3 (Nitrates) & Ammonia (NH_3) in a

stepwise manner which is then used by the plants.

Two types of Nitrogen Fixation

- Atmospheric Nitrogen Fixation can be classified into two main groups

1. **Symbiotic Nitrogen Fixation:** The process in which host plant & bacteria are mutually benefitted is known as symbiotic nitrogen fixation

E.g. The symbiotic bacterium Rhizobium is found in association with the root nodules of the leguminous plants such as beans, peas, gram, groundnut etc. In this association bacteria provide nitrites & nitrates to the plant while plant provide shelter to the bacteria. Means both are benefitted.

1. **Non Symbiotic Nitrogen Fixation:** Some of Nitrogen fixing organisms live independently of other organisms which are known as free living or non symbiotic nitrogen fixers. It was first reported by Kamen & Gest (1949). It is less efficient as compared to Symbiotic nitrogen fixation.

I. **Non Photosynthetic & Aerobic bacteria**: Azotobacter, Xanthomonas, Azospirillum, Azotococcus.

II. **Non photosynthetic & Anaerobic bacteria**: Clostridium, Methanococcus

III. **Photosynthetic & Anaerobic bacteria**: Chromatium,

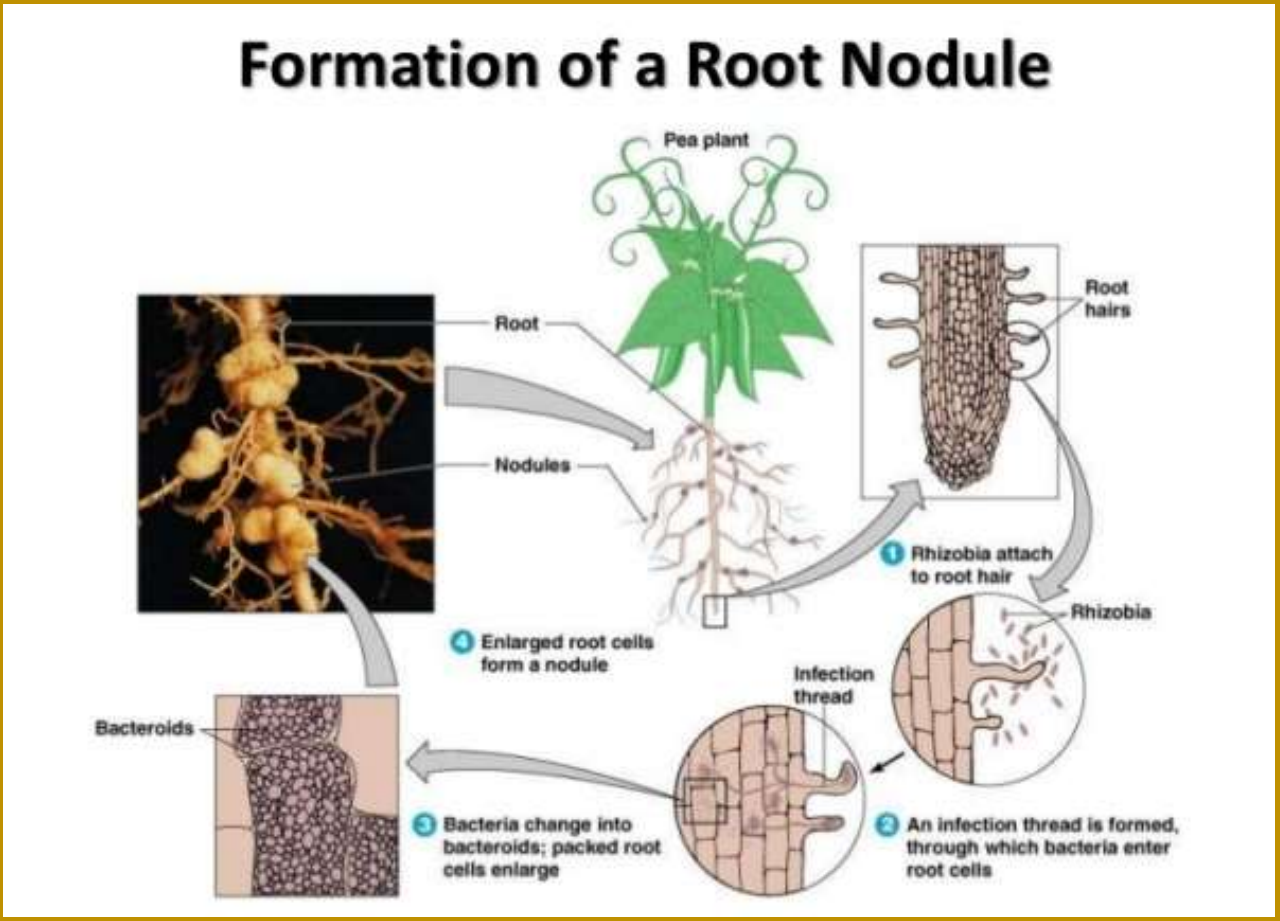
Rhodospirillum, Chlorobium

I. **Cyanobacteria (BGA):** Oscillatoria,

Gloeocapsa, Aphanocapsa, Calothrix.

Formation of Root Nodules

- Root nodules are sites of nitrogen fixation.



1. When root hairs of leguminous plants come in contact with the Rhizobium bacterium, It curls or become deformed.

Photo Courtesy : Google Images

2. At the site of curling the Rhizobia (bacteria) invade the root tissue.

3. Some of the bacteria within root tissue become enlarge to become membrane bound structures called bacteroids. These can not divide while some bacteria remain untransformed to facilitate further infection

4. The plant responds to this invasion by forming an infection thread made up of plasma membrane that grows inward from the infected cell of the host, separating the infected from the rest of the plant

5. Cell division now sets in, in the infected tissue leading to nodule formation.

Process of symbiotic nitrogen fixation in Legumes

- The root nodules of leguminous plants contain bacterium rhizobium.
- The root nodule contain one pigment called leghaemoglobin which gives a nodules a pinkish colour. Leghaemoglobin combine with oxygen and make anaerobic condition in root nodules.
- Leghaemoglobin is synthesis by bacteria as well as plant. Nodulin gene of plant express Globin protein while Haem (Iron containing compound) is formed by bacteria. Both haem & globin protein combine to formed Leghaemoglobin
- Rhizobium bacteria having one important enzyme for fixation of atmospheric nitrogen i.e. Nitrogenase (Which is code by Nif gene present in bacteria)
- Nitrogenase enzyme in anaerobic condition fix atmospheric nitrogen

Rhizobium (With Nitrogenase)

$N_2 + 3H_2 \longrightarrow 2NH_3$ (Highly Reduce Form)

BNF

↓ Nitrosomanas

NO_2 (Nitrite)

↓ Nitrobacter

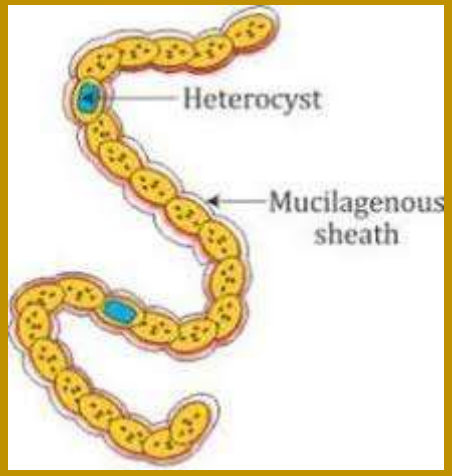
NO_3 (Nitrate)

Nitrification

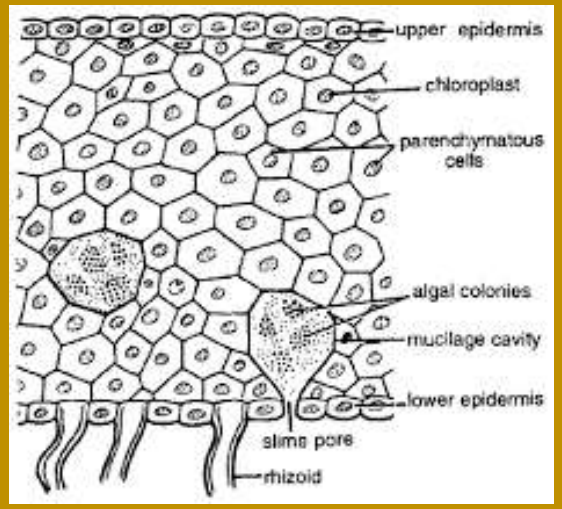
This Nitrate now available for plants

Symbiotic Nitrogen Fixation by Blue Green Algae (Cyanobacteria)

- Most of Blue Green Algae fix atmospheric nitrogen symbiotically in their Heterocyst's.
- Heterocyst's is different cell (Heteros means different) having very thick wall (cyst)
- The nitrogenase enzyme located in heterocyst which protected by oxygen by thick cyst.
- The coralloid roots of Cycas (Gymnosperm) show presence of Nostoc. Woody trees like Alnus & Casuarina show presence of Frankia. Anthoceros (Bryophyte) & Azolla leaves show presence of Nostoc.



Nostoc Filament



Symbiotic association Nostoc & Anthoceros

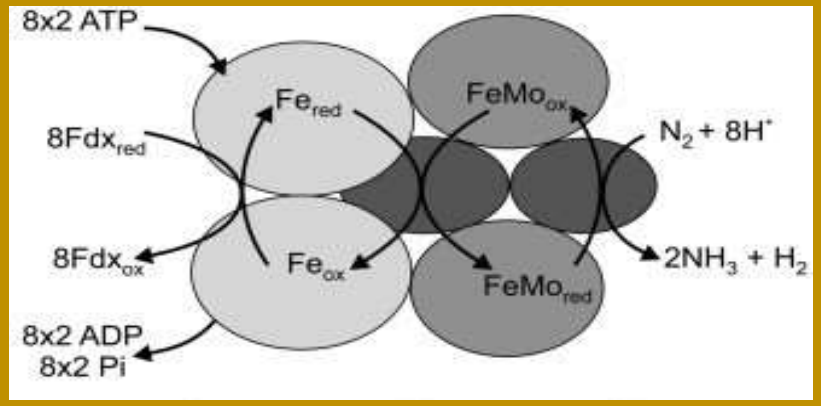
Photo Courtesy : Google Images

Some symbiotic Nitrogen fixers in different plants

Sr. No	Host Plant	Nitrogen Fixing Symbiont
1.	Sugarcane	Acetobacter
2.	Leguminous Plants	Rhizobium, Azorhizobium, Bradyrhizobium
3.	Alder & Casuarina	Frankia
4.	Miscanthus	Azospirillum
5.	Azolla (Water Fern)	Anabaena
6.	Anthoceros	Nostoc
7.	Cycas	Nostoc
8.	Gunnera	Nostoc

Nitrogenase Enzyme (Structure & Function)

- The Nitrogenase enzyme has two components, one containing Mo-Fe (Ferrous Molybdenum) protein & the other Fe (Ferrous) protein.
- Fe-Mo protein is bigger & consist of 4 subunits. Molecular weight is 180 to 235 kDa.



- The Fe-Protein is the smaller component composed of 2 subunits and has molecular weight 30 to 72 kDa.
- Each subunit contains an iron sulphur cluster that participates in redox reaction involved in the conversion of N2 to NH3
- The reaction catalysed is given below

$$N2 + 8H + 8e^- + 16\ ATP \longrightarrow 2NH_3 + H_2 + 16ADP + 16\ ip$$

Photo Courtesy : Google Images

Denitrification, Nitrification, Ammonification

- **Denitrification:** It is define as the process in which Nitrate is converted to gaseous compounds like nitric oxide, nitrous oxide & N2 by microorganisms.

  e.g. Pseudomonas, Bacillus.

  NO_3^- → NO^- → N_2O → N_2

  Nitrate — Nitric Oxide — Nitrous Oxide — Nitrogen

- **Nitrification:** The term nitrification refers to the conversion of ammonia (NH3) to Nitrite (NO2) & Nitrate (NO3). This is brought by Nitrifying bacteria.

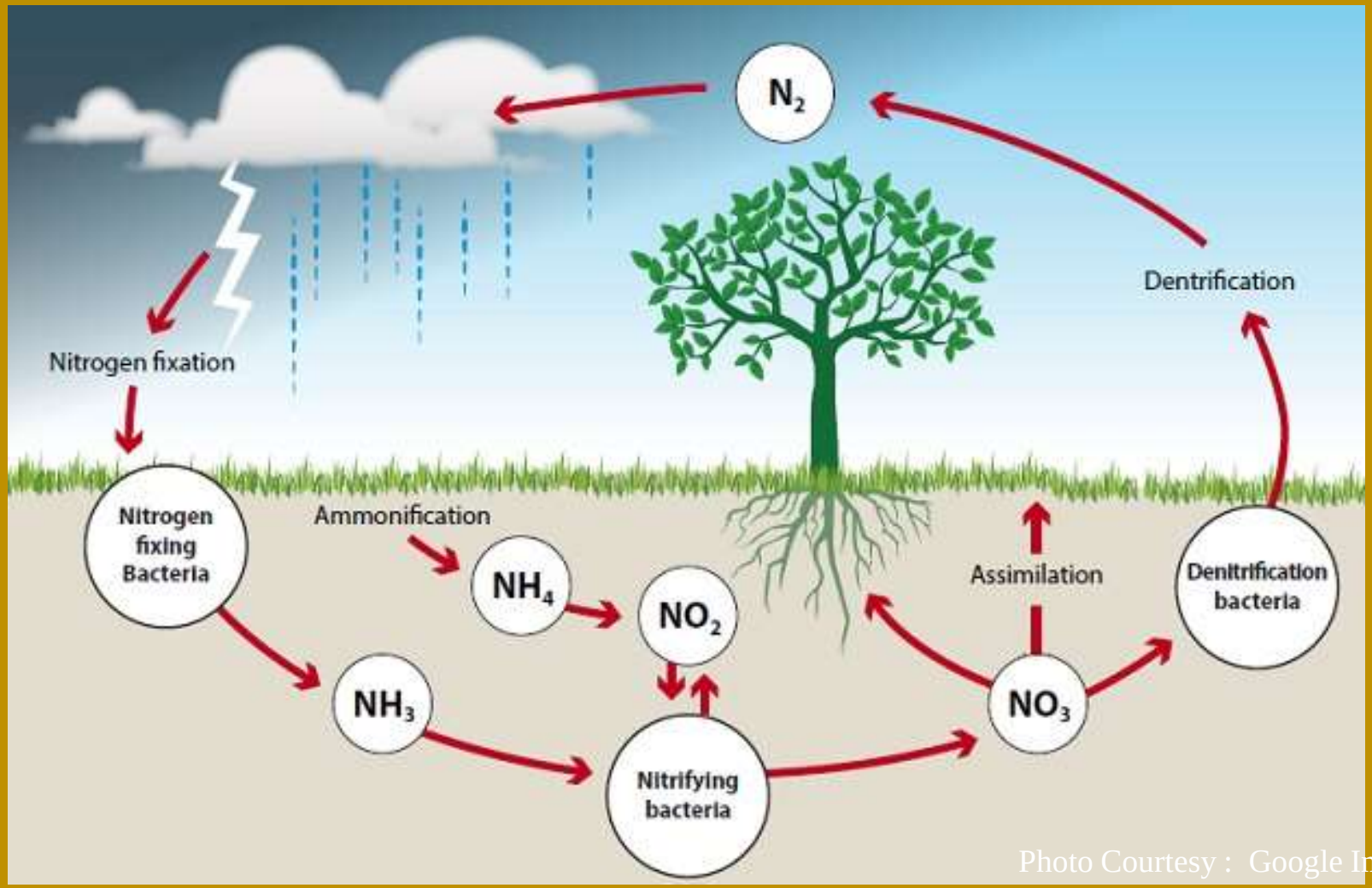
  e.g. Nitrosomonas, Nitrobacter etc.

  Ammonia (NH_3) —(Nitrosomonas, Nitrosococcus)→ Nitrite ions (NO_2) —(Nitrobacter)→ Nitrate ions (NO_4)

- **Ammonification:** The soil microorganisms such as bacteria & fungi convert the soil organic matter into ammonia through the process of mineralization, which is known as Ammonification.

Nitrogen Cycle

- Nitrogen cycle showing Nitrogen Fixation, Denitrification, Nitrification & Ammonification



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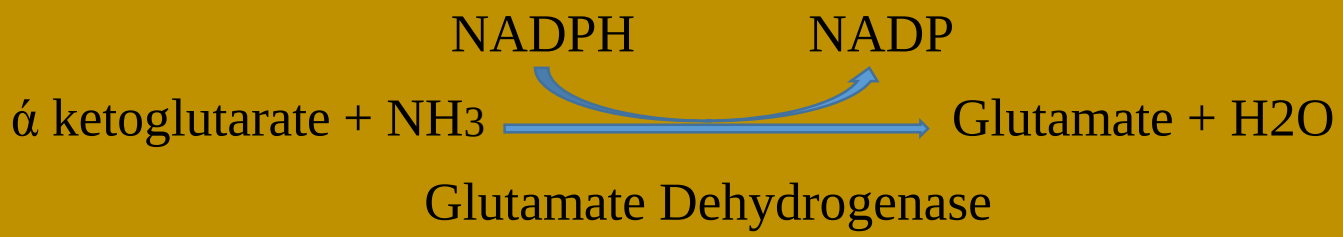
Reductive Amination & Transamination

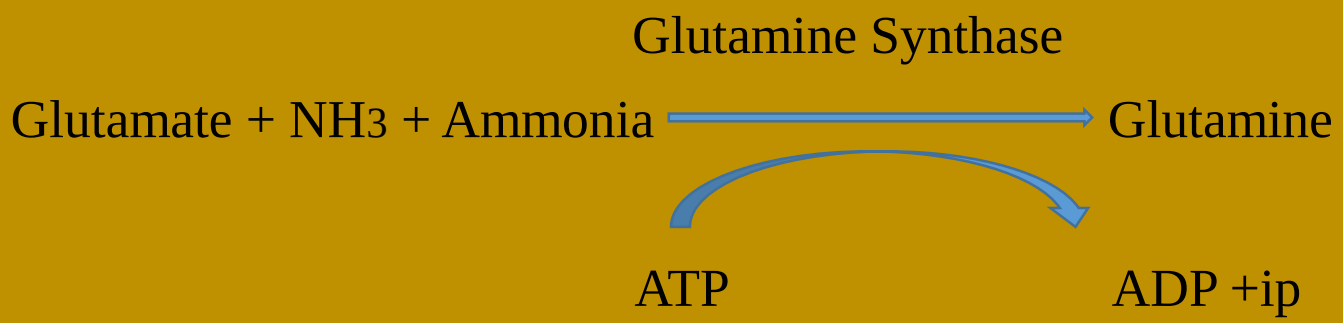
1. Reductive amination:

❖The reductive amination is the process in which ammonia is assimilated into plants by converting it into amino acids like glutamate, trough reduction process. The reaction involved in this process is given below



❖The glutamate is assimilated into plants even by converting it into its amide known as glutamine

❖



❖In this process ammonia reacts with ά ketoglutarate to form glutamic acid by reductive process. Hence it is known as reductive amination.

2. **Transamination**:

- Glutamic acid is the main amino acid from which other 17 amino acids are formed through transamination.
- Each amino acid consist one carboxyl group (-COOH) & one amino group (-NH). Transamination involves the transfer of amino group from one amino acid to the keto group of keto acid. The enzyme responsible for transamination is transaminase.
- Once nitrogen assimilated into glutamine & glutamate, nitrogen is incorporated into another amino acid via transamination reaction. The enzyme that catalyse these reactions are known as aminotransferases.
- An example is aspartate aminotransferase which catalyses the following reaction

- In the above reaction the amino group of glutamate is transferred to the carboxyl atoms of aspartate

Role of Nitrogen in plants

I. Nitrogen is important macronutrient because it is incorporated in Nucleic acids and proteins.

II. The amino acids are utilised in producing necessary enzymes & structural parts of the plants and can become a part of the stored proteins in the grain.

III. Some legume seeds such as Soyabeans & Peanuts contain high level of proteins and are among the most important agricultural sources of proteins in the world.

IV. Majority of secondary metabolites like alkaloids, glycosides, tannins, glycoproteins, lignin's etc. are derivatives of nitrogen

V. The purines & pyrimidine's the nitrogen bases of life molecules i.e. DNA are derived from nitrogen.

VI. Nitrogen is essential for synthesis of chlorophyll utilises the sunlight as an energy source to carryout essential plant functions including nutrient uptake.

VII. Nitrogen deficiency causes the yellowing or chlorosis of plant leaves.

VIII. Plant with nitrogen deficiency can also affect stand ability of crops as grain fill occurs & also plants become shorter & stunted in growth

REFERENCE

- **A reference book of Plant Physiology & Metabolism by Taiz & Zeiger**
- **A text book of Plant Physiology & Metabolism. S.Y.B.Sc Botany. Prashant & Nirali Prakashan**