

BIOLOGICAL NITROGEN FIXATION AND PLANT GROWTH HORMONES

34.1 INTRODUCTION

As you have learnt in Lesson 31, plants require certain macro and micro nutrients for their growth. Besides phosphorus and potassium, nitrogen is a key element for the nutrition of crop plants, and is termed as an essential nutrient for their growth. The nitrogen present in organic form in the soil is largely unavailable to the crop plants unless it is converted and brought into available form of ammonium (NH_4^+) and nitrate (NO_3^-) ions.

Chemical fertilizers like nitrogenous, phosphatic and potashic, have come to stay in the present century for increasing the output of crops so as to meet the ever increasing demands of the human population. The production of nitrogenous fertilizer is dependent on non renewable fossil fuels; and is highly energy consuming process. Moreover, there is a big gap between production and consumption of chemical fertilizers in our country; and we have to import large quantities of fertilizers causing a heavy drain on our limited foreign exchange reserves.

An alternative renewable source of nitrogen is the elemental nitrogen of atmosphere which contains 78 per cent nitrogen, which can be fixed (converted) into ammonical form by some nitrogen fixing microorganisms and plant system. This is the most inexpensive form of nitrogen which can be utilised by the plants; and it again goes back to the atmosphere through the nitrogen cycle which is operative in soil.

Plants and some soil microorganisms produce a variety of organic compounds which directly or indirectly affect plant growth. You will learn more about them in this lesson.

34.2 OBJECTIVES

After reading this lesson, you will be able to :

- explain the steps involved in nitrogen cycle
- describe the chemical and microbiological nitrogen fixation
- give reasons as to why we need nitrogen fixation
- list the organisms involved in biological nitrogen fixation
- explain the methods involved in increasing the biological nitrogen fixation (BNF)
- describe biofertilizers, their preparation and role in agriculture.
- explain plant growth hormones and their role in plant nutrition

34.3 NITROGEN CYCLE

Nitrogen cycle involves the following steps as explained below :

Ammonification

Nitrogen present in proteins and nucleic acids of plants is taken up by animals and these animals and plant cells after decay convert this nitrogen to ammonium as shown in step A.

Mineralization

Mineralization is the conversion of organic complexes into inorganic form.

In nitrogen mineralization part of soil organic matter (complexes) is decomposed and converted to inorganic ions like ammonium and nitrate which are used by plants. This is shown in step B.

Nitrification

Nitrification as denoted in "C" is the conversion of ammonium to nitrate by certain microorganisms.

Denitrification

Simultaneous to nitrification, under certain conditions denitrification also occurs as shown in step F whereby nitrate (NO_3^-) is converted to nitrogen gas or sometimes to nitric oxide (NO), and is lost to the atmosphere.

Nitrogen fixation

Opposite to denitrification there exists a reverse process of nitrogen fixation whereby atmospheric nitrogen is converted to ammonium form by certain microorganisms and plants to maintain the nitrogen balance in atmosphere.

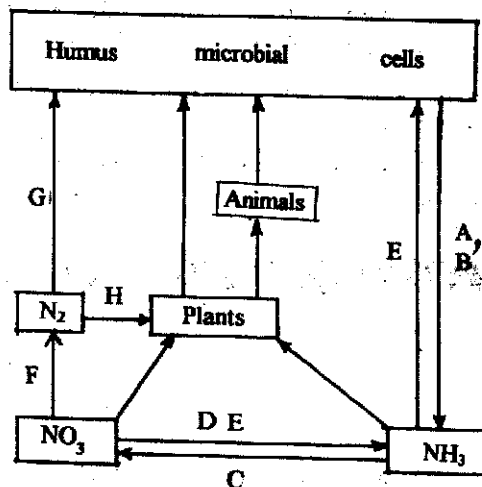
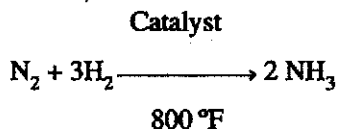


Fig. 34.1: Nitrogen Cycle

34.4 CHEMICAL NITROGEN FIXATION

This process came to be known only several years after the knowledge of biological nitrogen fixation. It involves the combination of nitrogen and hydrogen to form ammonia

in the presence of a catalyst at high temperature and pressure



In the area of chemical fixation, no breakthrough is yet visible to minimize the energy requirement to conventional Haber Bosch process for ammonia production. Therefore, the strategy for improving agriculture production in a country like ours should be to increase the inexpensive biological nitrogen fixation, and to make use of and popularise this practice among farmers. The ratio between biologically and chemically fixed nitrogen is 4 : 1.

34.5 WHY DO WE NEED BIOLOGICAL NITROGEN FIXATION ?

The simple answer to this question is that in our country there is a big gap between consumption and production of nitrogenous fertilizers. The requirement exceeds the fertilizer production. Hence we have to import fertilizers at a high price to meet the food requirements of our population. Moreover, due to high cost of machinery and rising cost of natural gas, it is difficult to erect new fertilizer plants.

Under such circumstances the biological fixation provides the answer to meet the nitrogen requirement of leguminous (you will learn about it in later section) and pulse crops through symbiotic nitrogen fixation and that of cereal crops like wheat and rice through free living nitrogen fixation.

INTEXT QUESTIONS 34.1

1. What are the major plant nutrients required by crop plants ?

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2. Define biological fixation of nitrogen.

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3. How is nitrogen balance maintained in the biosphere ?

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4. Explain mineralization, nitrification and denitrification.

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34.6 ORGANISMS INVOLVED IN NITROGEN FIXATION

The organisms involved in nitrogen fixation are:

1. The free living bacteria having ability to fix atmospheric nitrogen like *Azotobacter*,

Azospirillum, Beijerinckia, Clostridium.

2. Free living blue green algae fixing atmospheric nitrogen under rice cultivation.
3. Symbiotic nitrogen fixer *Rhizobium* which fixes nitrogen in symbiosis (association with leguminous plants).

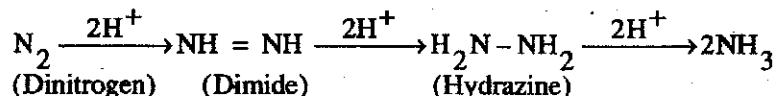
We shall now discuss all the three organisms and their nitrogen fixing ability.

34.7 FREE LIVING NITROGEN FIXATION

The fixation of atmospheric nitrogen by free living bacteria is also known as the non symbiotic nitrogen fixation. It is carried out by bacteria living freely in the soil, and without any symbiotic relationship fixes the nitrogen. The two most important such organisms are *Azotobacter* and *Azospirillum*.

34.7.1 Azotobacter

The number of *Azotobacter* cells in the Indian soils is rather low because of the lack of organic matter; and it is a limiting factor for their growth. The reaction in the enzymatic reduction of nitrogen to ammonia could be written as :



The ability to fix elemental nitrogen is a vital physiological characteristic of *Azotobacter* species. It is widely used as a bioinoculant in India for various crops like rice, wheat, mustard, cotton, vegetable crops; and higher yields have been obtained due to its inoculation. These increases in the yield are partly attributed to the growth promoting substances like indole acetic acid (IAA), gibberellins, vitamin B Complex, and the growth hormones produced by *Azotobacter* in addition to the fixed nitrogen made available to the plants.

34.7.2 Azospirillum

This is also a free living nitrogen fixer and has been isolated either from soil or roots of the crops. It is being used as a bioinoculant (bio-fertilizer) for different crops, like jowar, bajra, maize etc.

34.7.3 Blue green algae

Rice crop is largely grown in wet land conditions, with a layer of standing water which encourages the growth of algae. Blue green algae fixes nitrogen and can supply about 25-30 kg nitrogen/hectare to the rice crop. Some of the species which fix nitrogen are *Anabaena, Nostoc, Aulosira calothrix* etc.

34.8 SYMBIOTIC NITROGEN FIXATION

The word Symbiosis means a beneficial association between two partners. In the case of symbiotic nitrogen fixation it is an association between a legume plant and bacterium *Rhizobium*. In brief we will now discuss about both the partners.

34.8.1 Legumes

Legumes belong to the family leguminaceae and there are about 14000 species of legumes

known around the world. The role of legumes in enriching the fertility of soil was known through centuries; and the nodulated legumes contribute a good deal to the nitrogen fixed in the biosphere.

34.8.2 Rhizobium

This bacterium was isolated for the first time in 1838. It lives in soil and enters into symbiosis only with leguminous plants by infecting their roots and forming nodules on them. It lives inside the nodules and fixes atmospheric nitrogen for the plant. There are many species of *Rhizobium* present in the soil.

Can you guess whether the same *Rhizobium* can form nodules and fix nitrogen with all the leguminous plants. The answer is no. *Rhizobium* is very specific in choosing its symbiotic partner; and each *Rhizobium* species infects only limited number of legumes.

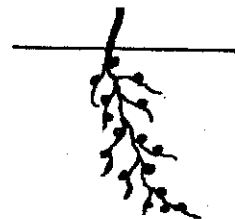


Fig. 34.2: Root nodules

It means that *Rhizobium* of pea group will only infect pea plant and fix nitrogen and not with the soybean plant. The soybean *Rhizobium* will only infect and form nodules on soybean plant and not on groundnut plant. This shows that *Rhizobium* is specific for each leguminous plant.

INTEXT QUESTIONS 34.2

1. What is free living nitrogen fixation ? List the organism involved in it.

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2. State the biofertilizer used in rice cultivation.

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3. Explain the process of Symbiosis.

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4. Can a single *Rhizobium* infect all leguminous plants ?

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34.9 BIOFERTILIZERS OR MICROBIAL INOCULANTS

The unique property of fixing atmospheric nitrogen by certain microorganisms, can be put to a great use by using them as a microbial inoculant to partially fulfil the nitrogen demand of various crops.

Microbial inoculants are preparations containing beneficial microorganisms in live state.

They are used for seed or soil application to improve soil fertility and plant growth. This is achieved by increasing the number and activities of desired microorganisms in the root region of plants. The *Rhizobium* inoculant for legumes and *Azotobacter*, and *Azospirillum* for non leguminous crops are becoming increasingly popular in India.

34.9.1 What are carrier and why do we need them ?

The microbial inoculant could either be in liquid form or solid form. As it is difficult to transport liquid culture to different places as desired by farmers it was practically convenient to send it in a solid medium called carrier material. The desired organism is put into sterilized carrier, placed in polythene bags and is sent to the user.

34.9.2 Selection of a suitable carrier

For supply of any microbial inoculant a suitable and cheap carrier material is required.

The ideal carrier should have high organic matter content, high water holding capacity, neutral pH, abundant supply and greater shelf life.

Various carrier materials used in India are peat soil from Nilgiri hills of Tamil Nadu, lignite, well decomposed farmyard manure, cowdung cake powder and charcoal – soil mixture. Amongst various materials used peat and charcoal – soil mixture are the two best materials. But as peat is expensive, mostly the finely ground charcoal – soil mixture in 2 : 1 ratio with 0.5% Potassium dihydrogen phosphate (KH_2PO_4) is used as a carrier for inoculant.

Eight kg of carrier is put in galvanised iron sheet trays and is sterilized at 15 lb. pressure for 3 hrs, on alternate 3 days to make it completely free of microorganisms. Two hundred gram of carrier to which desired bacterial culture broth (liquid) has been added; it is filled in polythene packets and incubated at $28 \pm 2^\circ\text{C}$ for 7 days. The microbial inoculant is now ready for field application. It has a shelf (active) life of 3 months.

34.9.3 Method for use of culture

Dissolve 100 gm of Sugar/gur in 1/2 to 1 litre water, boil it and cool. Empty the contents of culture packet into sugar/gur solution and make a slurry. Pour the seeds of the crop sufficient for one acre land in a suitable container and add the culture slurry. Continuously mix with a glass rod or clean hand so that culture is spread uniformly over seeds. Spread the inoculated seeds on a clean cloth or a gunny bag and dry in shade. Avoid exposure to direct sun light. Sow the dried seeds without delay. In case of transplantation crops such as paddy (rice), the roots of seedlings are dipped in the culture slurry for 2–3 hrs and then transplanted.

34.9.4 Crop responses to *Rhizobium* Biofertilizer

The beneficial effect of *Azotobacter* and *Azospirillum* inoculant on different crops has been discussed earlier in this chapter.

Response of *Rhizobium* inoculation on different grain legumes like urid, arhar, moong, gram, soybean, pea, groundnut has also been demonstrated in field trials at various locations in the country; and the yield increases ranging from 15 to 55 % have been obtained.

34.9.5 Importance of Biofertilizers

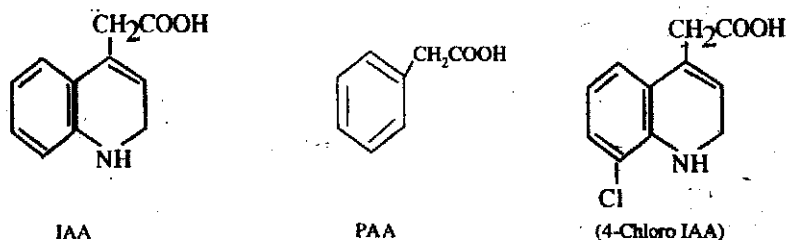
1. Nitrogenous and phosphate biofertilizers partially meet the nitrogen and phosphorus requirement of the crop plants, and by a judicious combination of chemicals and biofertilizers the full requirement of plant can be met under integrated nutrient management system.
2. They are inexpensive and rely on renewable resources like atmospheric nitrogen and microorganisms.
3. They do not cause any soil, water or atmosphere pollution as by the chemical fertilizer.
4. Due to a repeated and extensive use of chemical fertilizers the soil tends to loose its productivity; whereas by the use of biofertilizers soil is enriched due to the production of growth promoting substances.
5. Like synthetic fertilizers their production is not energy consuming.

34.10 PLANT GROWTH HORMONES

A plant growth hormone is an organic compound synthesised in one part of a plant and translocated (carried) to another part. In a very low (1 micro mole or even less) concentration it causes physiological changes in plants. There are three groups of well accepted hormones like, Auxins, Gibberellins and Cytokinins.

Auxins

These are the oldest and the best known plant growth regulators. The plants synthesise three auxins, of which Indole 3 acetic acid (IAA) is a true and important auxin hormone. The other two are 4-chloro indole acetic acid (4-Chloro IAA) and Phenyl acetic acid (PAA).



Besides these, now a days many synthetic auxins are also available like 2, 4-dichlorophenoxy acetic acid (2, 4 - D), 2, 4, 5-trichlorophenoxy acetic acid (2, 4, 5 - T) and naphthalene acetic acid (NAA) etc. Auxins promote the elongation of root system of plants and also the root development on stems. *Rhizobium* also produces IAA, and causes elongation and curling of root hairs.

Gibberellins

More than sixty gibberellins have been discovered in plants and microorganisms (fungi and algae). All gibberellins have 19 or 20 carbon atoms grouped in a total of either four or five ring systems, and all have one or more carboxylic (COOH) groups. They are abbreviated as GA₁, GA₂ and GA₃. All could properly be referred to as gibberellic acids, but GA₃ has been studied much more than any others, so it is commonly known as gibberellic acid.

They play an important role in the growth of many plants by increasing their roots and leaves. They also promote germination of dormant (which germinate taking long time) seeds and buds. They help in the flowering of plants. Gibberellins are sprayed on fruits

plants like seedless grapes and oranges for better quality fruit. They are now commercially used to increase the sugarcane growth.

Cytokinins

These are the compounds present in plants; and they take part and help in the activities of plants. One such compound is kinetin. Cytokinins promotes cell division and formation of different parts of a plant. They also delay the senescence (becoming old) of plants and help in the growth of stem and roots.

34.11 WHAT YOU HAVE LEARNT

- The various steps involved in nitrogen cycle are ammonification, mineralization, nitrification, denitrification and nitrogen fixation.
- Chemical nitrogen fixation involves the combination of nitrogen and hydrogen to form ammonia in the presence of a catalyst at high temperature.
- Biological nitrogen fixation helps to meet the nitrogen requirement of leguminous and pulse crops.
- The organisms involved in biological nitrogen fixation are *Azotobacter*, *Azospirillum*, *Beijerinckia*, *Clostridium* and *Rhizobium*
- Biofertilizers or microbial inoculants are preparations containing beneficial microorganism in live state.
- Biofertilizers are used for seed or soil application to improve soil fertility and plant growth.
- Plant growth hormone is an organic compound synthesised in one part of a plant and translocated to another part.

34.12 TERMINAL QUESTIONS

1. State whether the following statement are true (T) or false (F)
 - a. Atmosphere contains 78% nitrogen T/F
 - b. Mineralization is the conversion of inorganic to organic form. T/F
 - c. Denitrification process leads to nitrogen loss. T/F
 - d. Crop plants use nitrogen in ammonium or nitrate form. T/F
 - e. Biological nitrogen fixation is a highly energy consuming process. T/F
 - f. *Rhizobium* is a free living nitrogen fixer. T/F
 - g. *Azotobacter* produces growth promoting substances in soil. T/F
 - h. *Azospirillum* inoculant is used for rice cultivation. T/F
 - i. 14000 species of legumes are known around the world. T/F
 - j. *Rhizobium* lives inside the nodule and fixes nitrogen. T/F
2.
 - a. Define biofertilizers.
 - b. What are the properties of good carrier material ?
 - c. List the carries used for microbial inoculants.

- d. List the crops for which microbial inoculants are available.
 - e. State the importances (any two) of biofertilizers
- 3.
- a. What are the plant growth hormones ?
 - b. List different plant hormone.
 - c. How do they help the plants ?

CHECK YOUR ANSWERS:

KEY TO INTEXT QUESTIONS 34.1

1. The major plant nutrients required by crop plants are :
 - (a) Nitrogen
 - (b) Phosphorus
 - (c) Potassium
 - (d) Sulphur
 - (e) Carbon
2. Biological nitrogen fixation is the microbial conversion of elemental atmospheric nitrogen to ammonia.
3. The biological nitrogen fixation converts nitrogen to ammonia form. This ammonia is converted to Nitrate form through the process of nitrification; and this nitrate is converted back to nitrogen gas by process of denitrification.
4. MINERALISATION : It is conversion of organic nitrogen to inorganic form
- NITRIFICATION : It is microbial conversion of ammonia to nitrate
- DENITRIFICATION : It is microbial conversion of nitrate to nitrogen and nitric oxide under certain soil conditions

KEY TO INTEXT QUESTIONS 34.2

1. Non symbiotic nitrogen fixation is known as free living nitrogen fixation. The organisms involved are
 - (a) *Azotobacter*
 - (b) *Azospirillum*
 - (c) *Beijerinckia*
 - (d) Blue green algae
2. Blue green algae is a biofertilizer used for rice cultivation. It grows in standing water in the rice field and fixes about 25–30 kg nitrogen/ha.
3. Symbiosis means a beneficial association between two partners. In this process both the partners are benefited by the action of each other.
4. No. The *Rhizobium* is specific for certain legumes. One *Rhizobium* strain may infect three or four leguminous plants belonging to the same inoculation group but not all the legumes.

TERMINAL EXERCISE

1. a. True b. False c. True d. True e. False f. False g. True h. False i. True j. True.
2. (a) Biofertilizers are carrier based preparations containing beneficial microorganisms in live state used for seed or soil application to improve plant growth by increasing number and activities of desired microorganisms in the root region.
(b) A good carrier material should have
 - (i) high organic matter content
 - (ii) high water holding capacity
 - (iii) neutral pH
 - (iv) abundance and cheapness of the material(c) The carriers used for preparation of microbial inoculant are :
 - (i) Peat soil
 - (ii) Lignite
 - (iii) well decomposed farmyard manure
 - (iv) ground charcoal and soil mixture in the ratio of 2 : 1(d) Microbial inoculants are available for many legume crops, pulse crops like pea, groundnut, arhar, moong, frenchbean, soybean, urid, gram etc. and cereal crops like wheat, rice, maize, jowar, millet and vegetables.
(e) (i) They supply nitrogen and phosphorus to the crop thereby reducing the application of chemical fertilizers
(ii) They are inexpensive and ecofriendly (do not cause soil and atmosphere pollution)
3. (a) These organic compounds are synthesised by plant and microorganisms and in very small concentration help plant growth.
(b) Auxins, Gibberellins and Cytokinins are three main groups of plant hormones.
(c) They help in the overall growth and in the root development and flowering of plant. They help in the germination of dormant seeds and buds. Gibberellins are sprayed on fruit plants for better fruit quality.