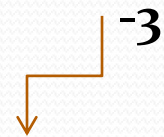




# **BIOLOGICAL NITROGEN FIXATION**

# INTRODUCTION

- Nitrogen is the most common amongst all soil nutrients required by the plants in a larger quantity.
- But the di-nitrogen gas( $N_2$ ), i.e. The atmospheric nitrogen, is not available directly to them in any usable form.
- Nitrogen in the form of Dinitrogen( $N_2$ ) makes up 80% of the air we breathe but is essentially inert due to the triple bond ( $N\equiv$ ).
- Plants are able to use nitrogen in the form of nitrate( $NO_3^-$ ) or ammonia ( $NH_3^+$ ), but these compounds are present in limited supply in the soil.



Oxidation state of N

# BIOLOGICAL NITROGEN FIXATION

- The utilization of atmospheric di-nitrogen gas(N<sub>2</sub>) as a source of cell nitrogen by way of its reduction to ammonia by a biological agents is called *biological nitrogen fixation*.
- The general equation of N<sub>2</sub>- fixation is as follows:



# MODES OF NITROGEN FIXATION

- These are following three principal modes adopted by a variety of prokaryotic microbes to reduce atmospheric nitrogen to ammonia :
1. **Non- symbiotic**
  2. **Associative symbiotic**
  3. **Symbiotic**

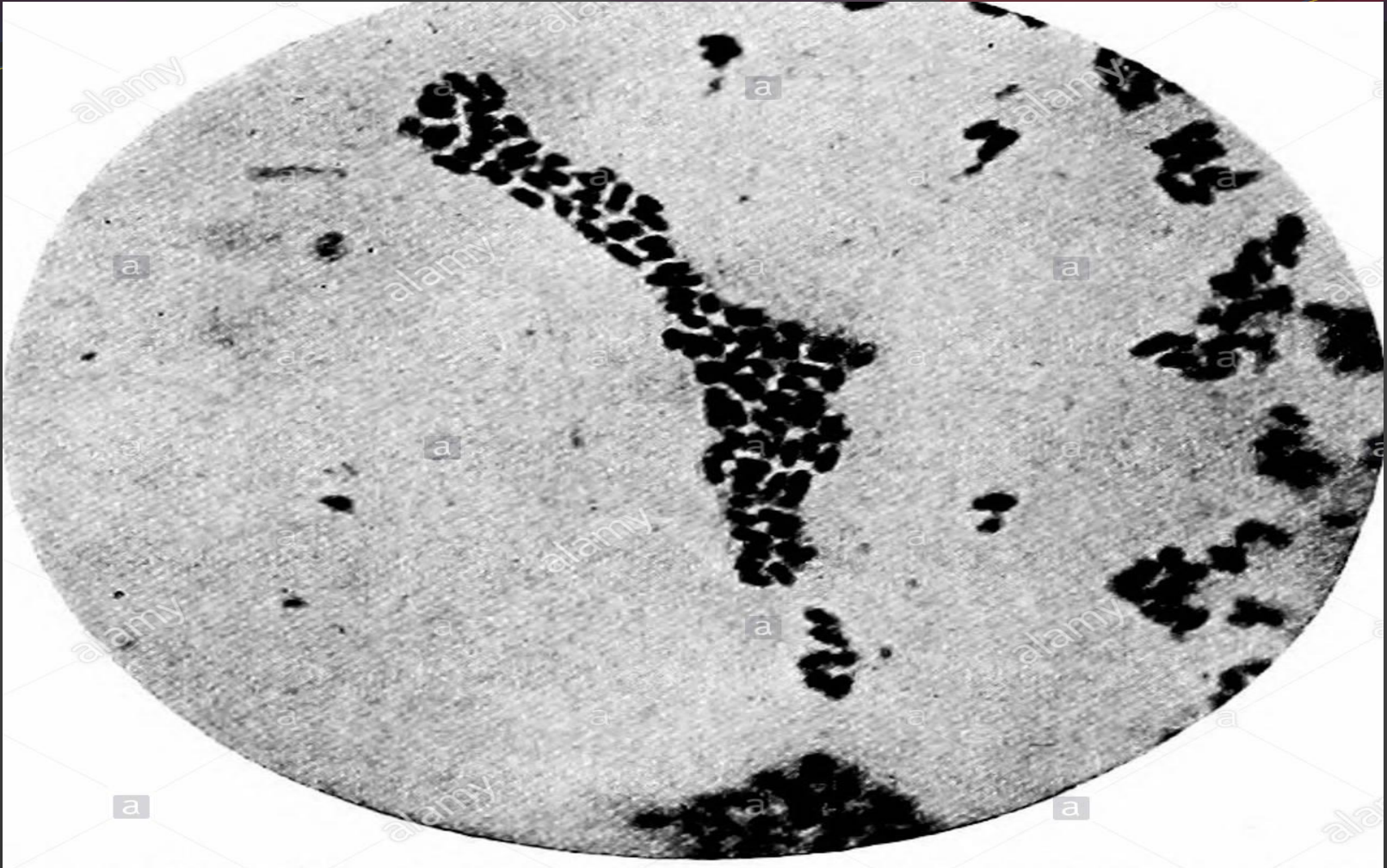
# 1. NON- SYMBIOTIC NITROGEN FIXATION

- This type of biological nitrogen fixation is effected by those micro-organisms, which live freely & independently in the soil.
- A large number of bacteria & some cyanobacteria are thought to be capable of non-symbiotic N<sub>2</sub> – fixation.

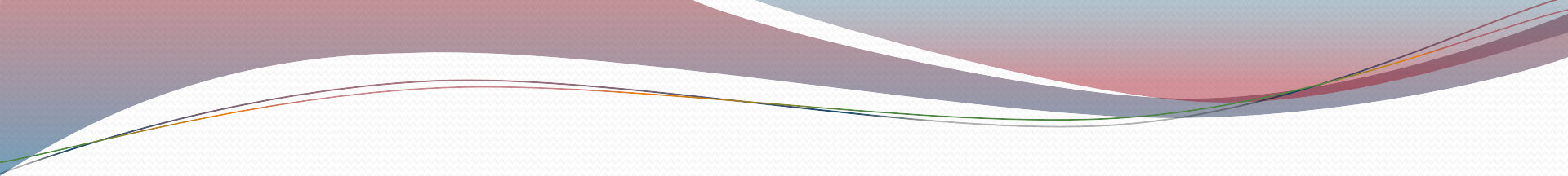
*Azotobacter, Azomonas, Azotococcus, Mycobacterium spp., Thiobacillus ferrooxidans, E.coli, Clostridium spp., etc ...*

exemplify the bacteria (free living)capable of non- symbiotic biological nitrogen fixation.

*Azotobacter spp.(aerobic)* are the main nitrogen fixing free living bacteria.



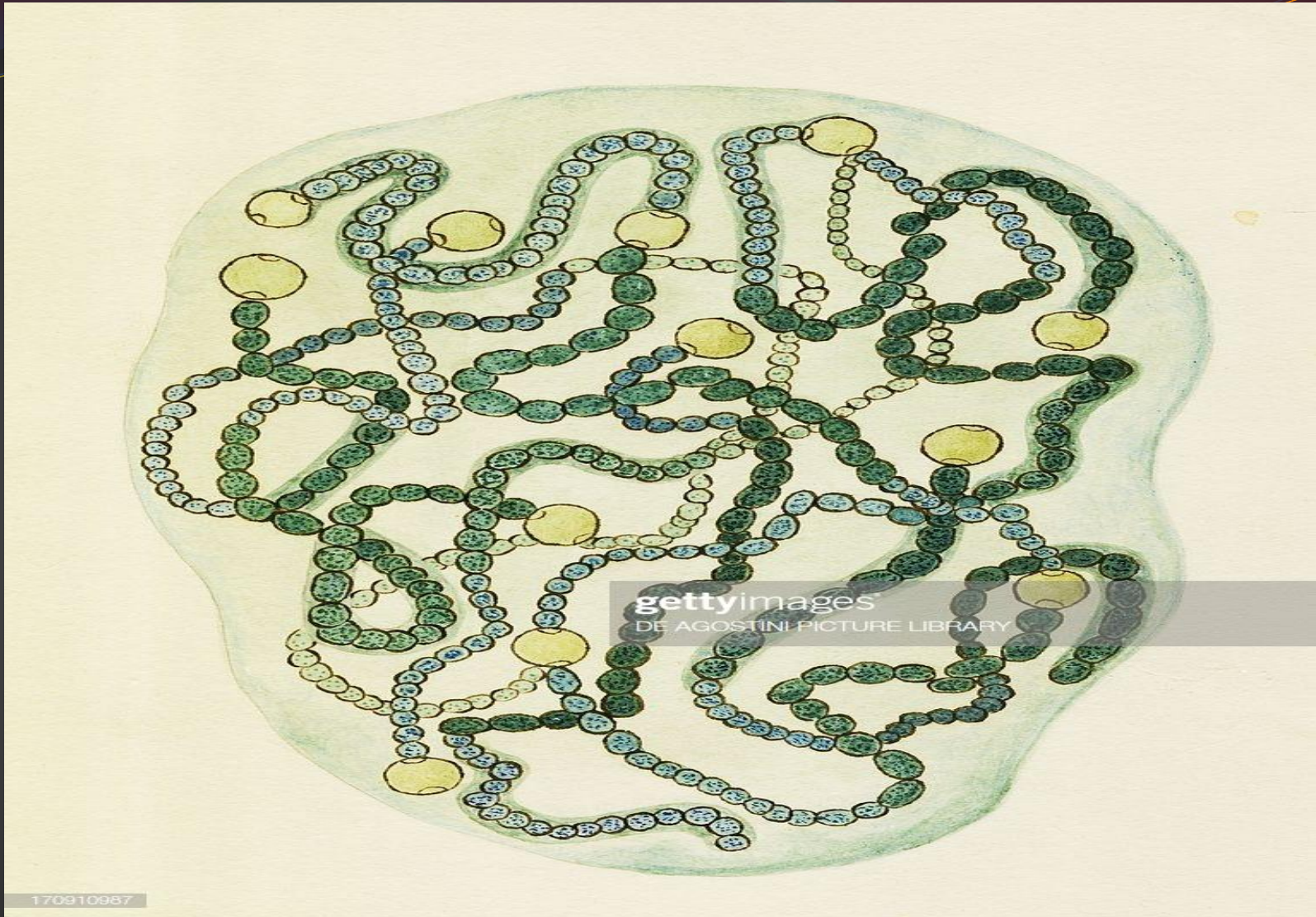
**Azotobacter** species cells, stained with Heidenhain's iron hematoxylin, \*1000

- 
- *Clostridium spp.* (anaerobic) stands the next.
  - The free-living cyanobacteria are considered to be fairly important **nitrogen-fixers**.
  - Cyanobacteria help in **maintaining the fertility and productivity of rice field**.
  - *Anabena* and *Nostoc* are the good example of **nitrogen fixing cyanobacteria**.



**Anabaena** flos-aquae, nitrogen fixing fresh water cyanobacteria, **seen under a microscope.**

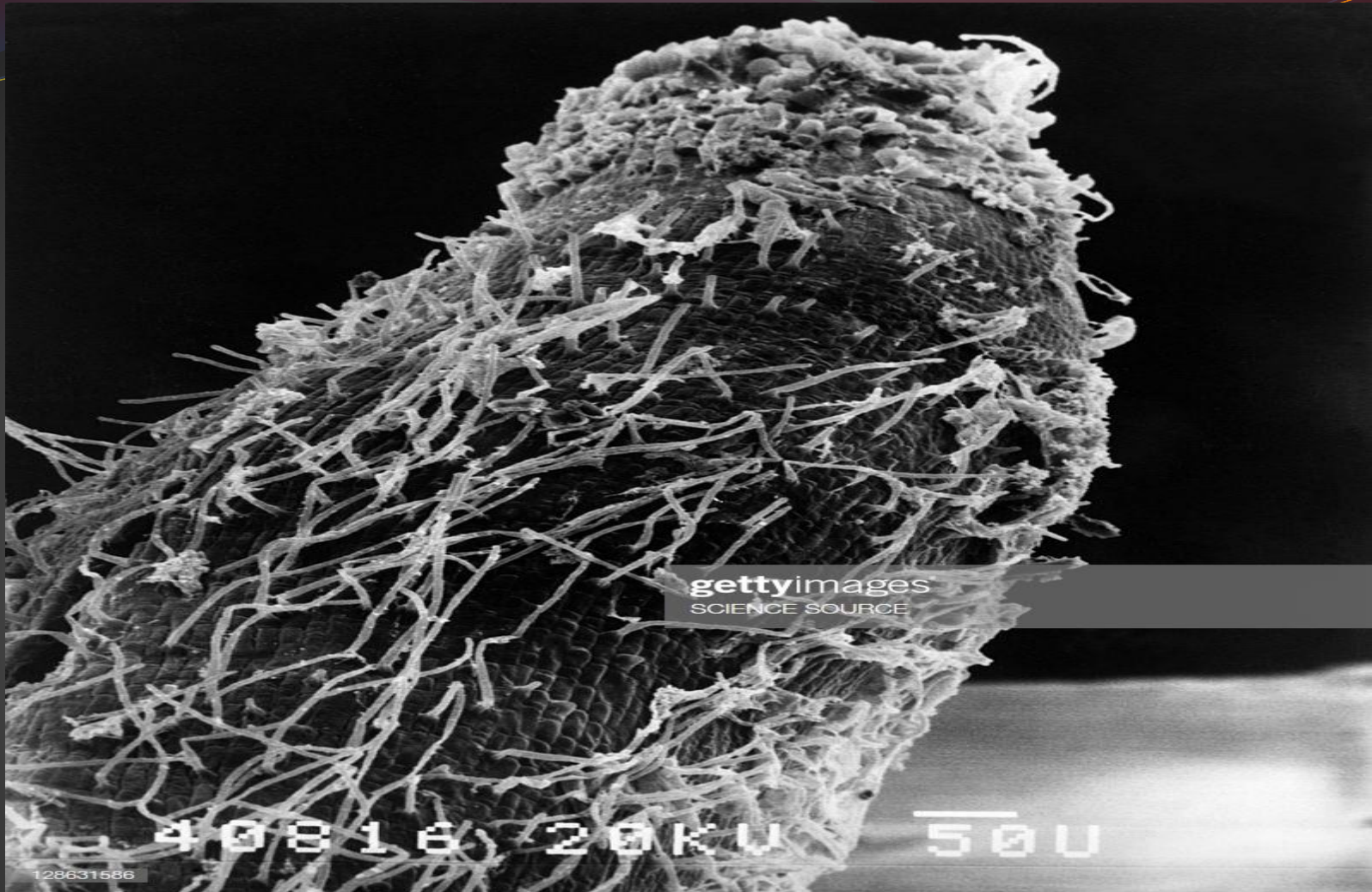




*Nostoc linckia*, cyanobacteria or fresh water nitrogen-fixing blue-green algae seen under a microscope.

# 2. ASSOCIATIVE SYMBIOTIC NITROGEN FIXATION

- **Associative symbionts** are recent added to the area of biological nitrogen fixation .
- They are usually found association with the **roots of grasses and cereal plants.**
- *No nodules are formed* here , instead they are formed by **symbiotic association.**
- These bacteria grow in the *rhizosphere* in **close contact with the roots** , sometimes invade the **outer cortical region and fix nitrogen.**
- *Azospirillum brasilense, pseudomonas azotogenesis, enterobacter, bacillus, klebsiella, etc.*

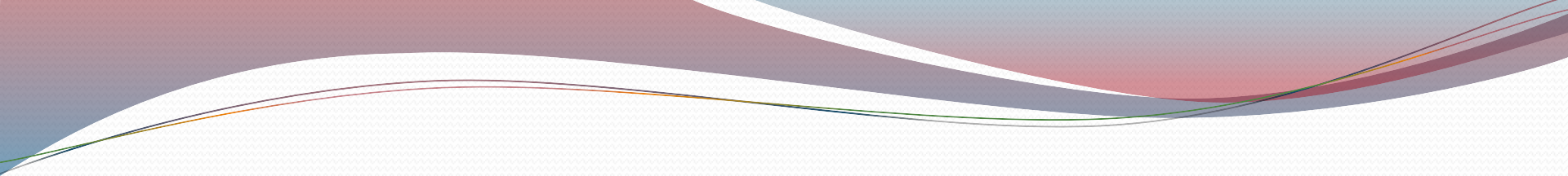


A scanning electron micrograph of a **root tip from a sorghum** plant with kidney bean shaped bacteria (***Azospirillum brasilense***) on its surface.

# 3. SYMBIOTIC NITROGEN FIXATION

## I. THROUGH NODULE FORMATION IN LEGUMES

- Symbiotic association is the most interesting and important of the various plant-bacteria interaction; the plants being legumes and the bacteria *Rhizobium*, *sinorhizobium*, *Azorhizobium*.
- These bacteria are **gram negative motile rods**.
- Infection of the roots of a leguminous plant (e.g., **soybeans, clover, alfalfa, beans, peas**) with the appropriate species of one of these genera leads to formation of root nodules that are able to **convert atmospheric nitrogen to ammonia** by nitrogen-fixing mechanism.

- 
- *Azorhizobium* have **agricultural significance** as it leads to a greater quantitative **enhancement of combined nitrogen** in the soil.
  - About **90% of all legumes** species become nodulated.
  - A group of Rhizobium strains capable of infecting a group of related legumes is referred to as “**Cross- inoculation group.**”



Nitrogen-fixing bacteria ([Rhizobium](#)) nodules on Soyabean roots.

## II. THROUGH NODULE FORMATION IN NON-LEGUMINOUS PLANTS

- Recently it has been found that certain non-leguminous plants form nodules to fix nitrogen.
- The best known plant, in **temperate region**, is **alder (*Alnus spp.*)**; the bacterium involved is *actinomyces*.
- Other non-leguminous plants that have root nodules are ***casuarina* in tropics and *Hippophae*** which colonizes sand dunes.



Green alder (*Alnus crispa*) leaves and catkins.



### III. WITHOUT NODULATION

- Non- nodulating nitrogen fixing association are formed between **cynobacteria and various plant.**
- The **cyanobacterium *Anabaena Azollae*** forms Symbiotic with ***Azzola*.**
- **Nostac** is found in the stem of ***Gunnera Macrophylla*.**
- ***Azobacter paspali*** develops colonies below mucilaginous root sheath of ***paspalum notatum***
- ***Spirillum notatum*** lives associated in or around the **roots of Digitaria , maize ,etc.**

# ENZYMES OF NITROGEN FIXATION :-

1. NITROGENASE

2. HYDROGENASE

# 1. NITROGENASE

- It is a complex enzyme system that is chiefly responsible for **N<sub>2</sub>-fixation in microbes**.
- It is very sensitive to oxygen and must be protection from oxygen-inactivation within the cell.
- In legume nodules, the protection against oxygen is provided by **leghaemoglobin**, whereas in many **cyanobacteria** it is achieved by a special structure called the *heterocyst*.
- Nitrogenase consist of two major protein components:
  - I. Dinitrogenase (MoFe-protein)*
  - II. Dinitrogenase reductase (Fe-protein)*

## 2. HYDROGENASE

- Many **diazotrophs** evolve hydrogen during  $N_2$ -fixation, which in turn **inhibits the  $N_2$ -fixation reaction**.
- This inhibitory action of  $H_2$  is **overcome by hydrogenase enzyme**.
- Use of  $H_2$  by **purple sulphur bacteria** was for the **first time** reported by **ROELOFSON** in **1935**.
- He stated that “**the hydrogen( $H_2$ ) produced by nitrogenase enzyme is recycled in nitrogen-fixing system again by the enzyme hydrogenase.**”

@A S PHOTOGRAPHY

THANK YOU

