NITROGEN

FIXATION

M.Uma Maheshwari,
Reg No. 13APBO10
II M.Sc Botany

ROLE OF NITROGEN IN PLANTS

- Major substance in plants next to water
- Building blocks
- Constituent element of
  - Chlorophyll
  - Cytochromes
  - Alkaloids
  - Many vitamins
- Plays important role in metabolism, growth, reproduction and heredity

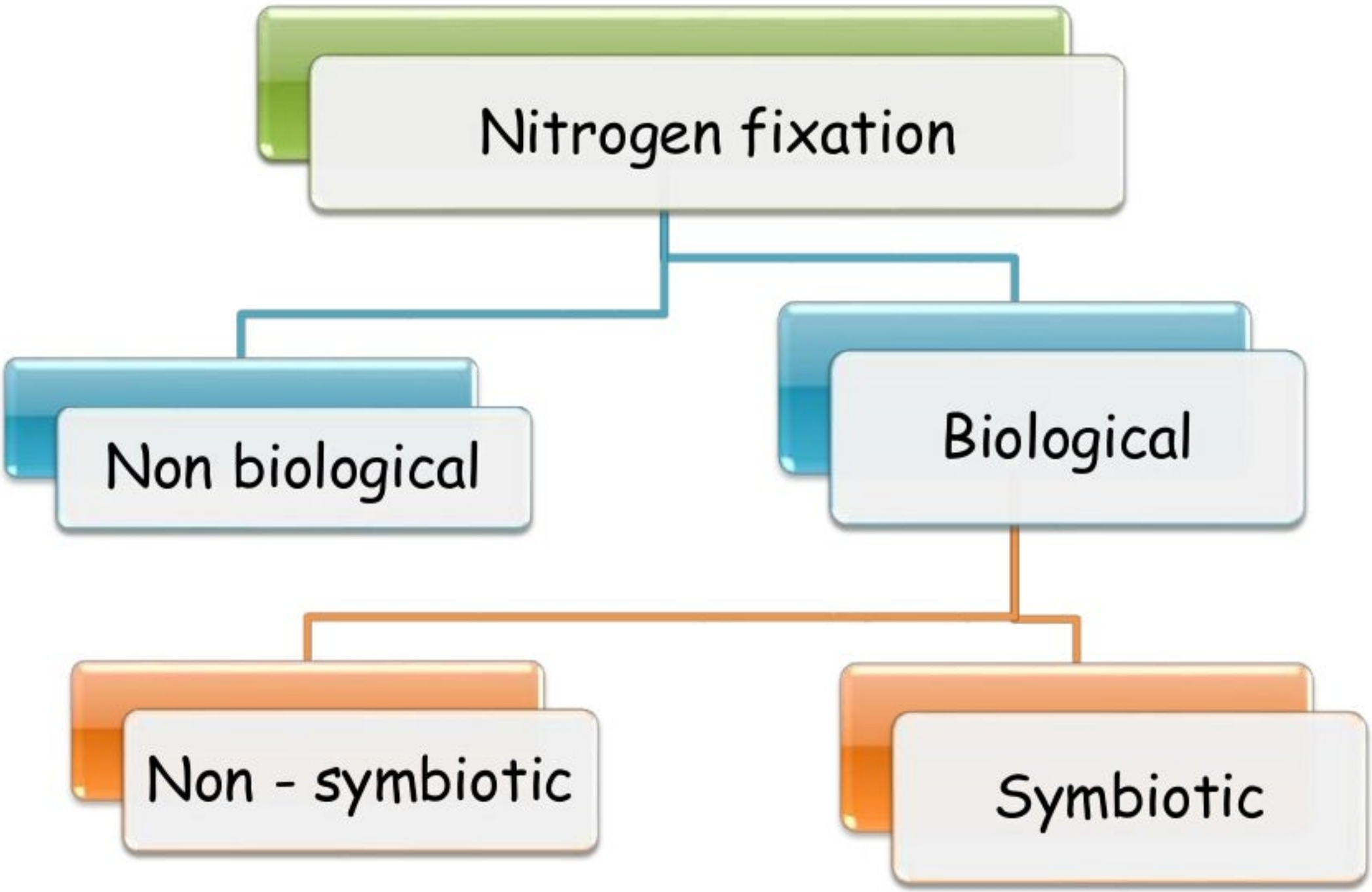
SOURCES OF NITROGEN

- Atmospheric Nitrogen
  - 78% of atmosphere
  - Plants cannot utilize this form
  - Some Bacteria, Blue Green Algae, leguminous plants
- Nitrates, Nitrites and Ammonia
  - Nitrate is chief form
- Amino acids in the soil
  - Many soil organisms use this form
  - Higher plants can also taken by higher plants
- Organic Nitrogenous compounds in insects
  - Insectivorous plants

NITROGEN FIXATION

- The conversion of free nitrogen into nitrogenous salts to make it available for absorption of plants

TYPES OF NITROGEN FIXATION



NON BIOLOGICAL FIXATION

- The micro-organisms do not take place
- Found in rainy season during lightning

1. $N_2 + O_2 \xrightarrow{\text{lightning}} 2\,NO$
   (Nitric oxide)
2. $2NO + O_2 \xrightarrow{\text{oxidation}} 2NO_2$
   (Nitrogen per oxide)
3. $2NO_2 + H_2O \longrightarrow HNO_2 + HNO_3$
4. $4NO_2 + 2H_2O + O_2 \longrightarrow 4HNO_3$
   (Nitric acid)
5. $CaO + 2HNO_3 \longrightarrow Ca(NO_3)_2 + H_2O$
   (Calcium nitrate)
6. $HNO_3 + NH_3 \longrightarrow NH_4NO_3$
   (Ammonium nitrate)
7. $HNO_2 + NH_3 \longrightarrow NH_4NO_2$
   (Ammonium nitrite)

BIOLOGICAL FIXATION

- Fixation of atmospheric Nitrogen into nitrogenous salts with the help of micro-organisms
- Two types
  - Symbiotic
  - Non-symbiotic

NON-SYMBIOTIC

- Fixation carried out by free living micro-organisms
- Aerobic, anaerobic and blue green algae
- Bacteria: special type (nitrogen fixing bacteria) types -
  - Free living aerobic : *Azotobacter, Beijerenckia*
  - Free living anaerobic : *Clostridium*
  - Free living photosynthetic : *Chlorobium, Rhodopseudomonas*
  - Free living chemosynthetic :*Desulfovibro,Thiobacillus*

CONTD..

- Free living fungi: yeasts and *Pillularia*
- Blue green algae:
  - unicellular – *Gloeothece, Synechococcus*
  - Filamentous (non heterocystous) -*Oscillatoria*
  - Filamentous (heterocystous) – *Tolypothrix, Nostoc, Anabaena*

SYMBIOTIC

- Fixation of free nitrogen by micro-organisms in soil living symbiotically inside the plants
- 'Symbiosis' – coined by DeBary
- Three categories
  - Nodule formation in leguminous plants
  - Nodule formation in non-leguminous plants
  - Non nodulation

NODULE FORMATION IN LEGUMINOUS PLANTS

- 2500 spp. Of family leguminosae (*Cicer arientium, Pisum, Cajanus, Arachis*) produce root nodules with *Rhizobium* spp.
- They fix Nitrogen only inside the root nodules
- Association provides-food and shelter to bacteria

  -bacteria supply fixed nitrogen to plant
- Nodules may buried in soil even after harvesting – continue nitrogen fixation

NODULE FORMATION IN NON-LEGUMINOUS PLANTS

- Some other plants also produces root nodules
  - *Causuarina equisetifolia – Frankia*
  - *Alnus – Frankia*
  - *Myrica gale – Frankia*
  - *Parasponia – Rhizobium*
- Leaf nodules are also noted
  - *Dioscorea, Psychotria*
- Gymnosperms – root – *Podocarpus,*
  - leaves – *Pavetta zinumermanniana, Chomelia*

NON-NODULATION

- Lichens - *cyanobacteria*
- *Anthoceros* - *Nostoc*
- *Azolla* – *Anabaena azollae*
- *Cycas* – *Nostoc* and *anabaene*
- *Gunnera macrophylla* - *Nostoc*
- *Digitaria, Maize and Sorghum* – *Spirillum notatum*
- *Paspalum notatum* – *Azotobacter paspali*

SYMBIOTIC NITROGEN FIXATION

- Small, knob-like protuberances-root nodules
- Size and shape varies
- Spherical, flat, finger-like or elongated
- From Pin head to one centimeter in size
- Various spp. Of Rhizobium noted
- Named after the host plant
  - Pea – Rhizobium leguminosarum
  - Beans – R. phaseoli
  - Soyabeans – R. japonicum
  - Lupins – R. lupini
- Two types of Rhizobium-
  - Bradyrhizobium – slow growing spp.
  - Rhizobium - fast growing spp.

RHIZOBIUM

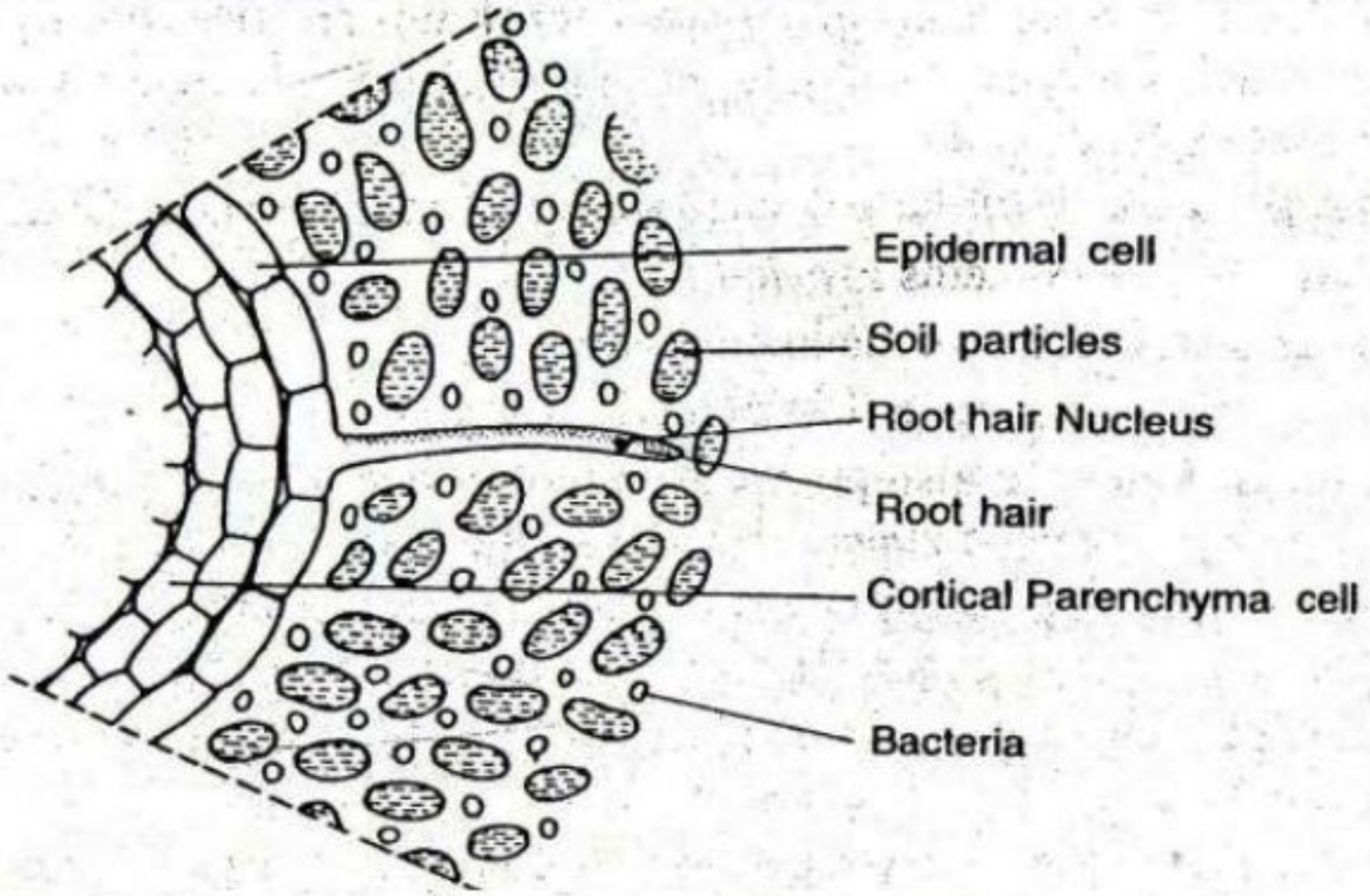
- Gram negative
- Non spore forming
- Micro-aerobic
- Show a degree of specificity
- The two partners (Bacteria and Host) recognized by chemical substance LECTINS - phytoagglutinins (carbohydrate containing plant protein)

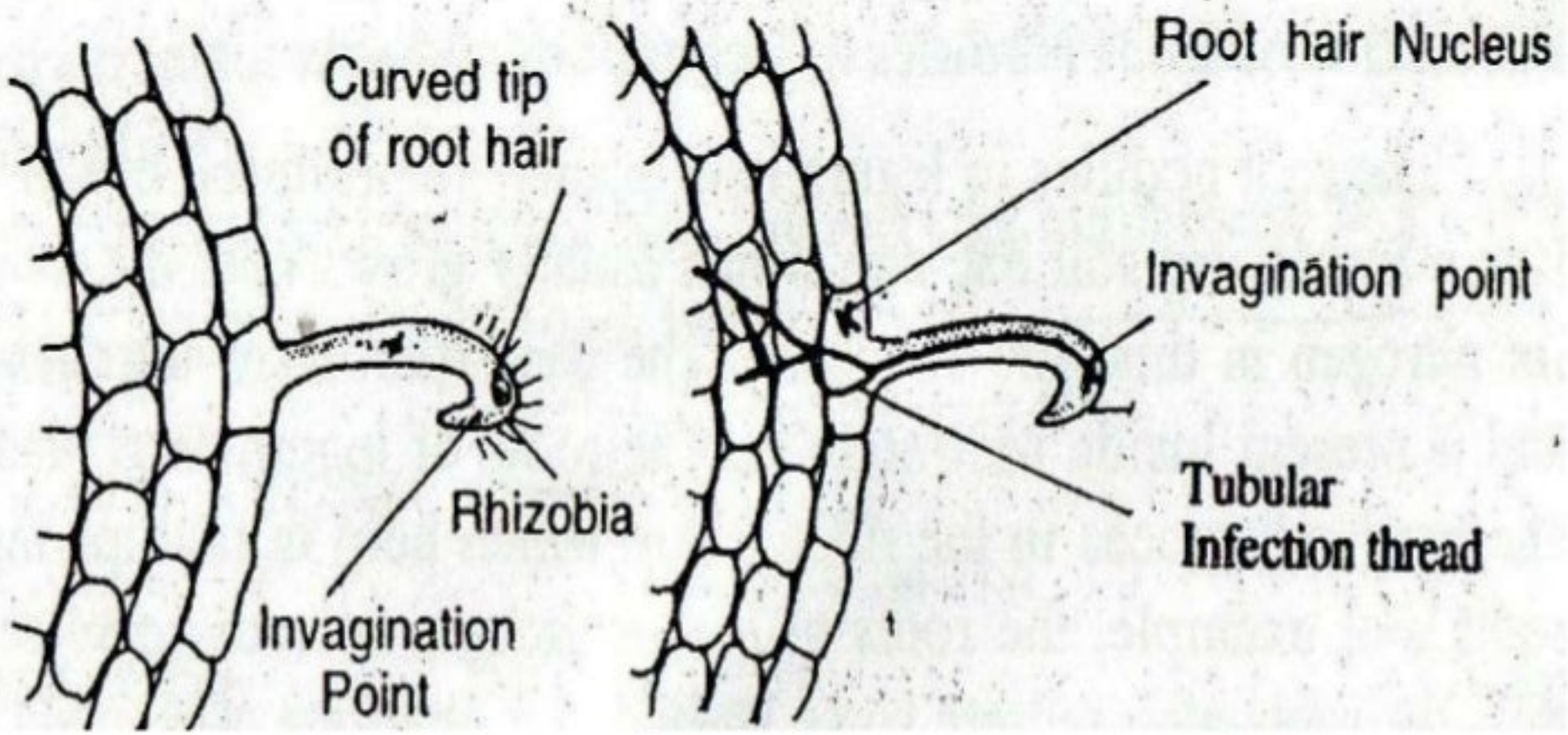
FORMATION OF ROOT NODULES IN LEGUMES

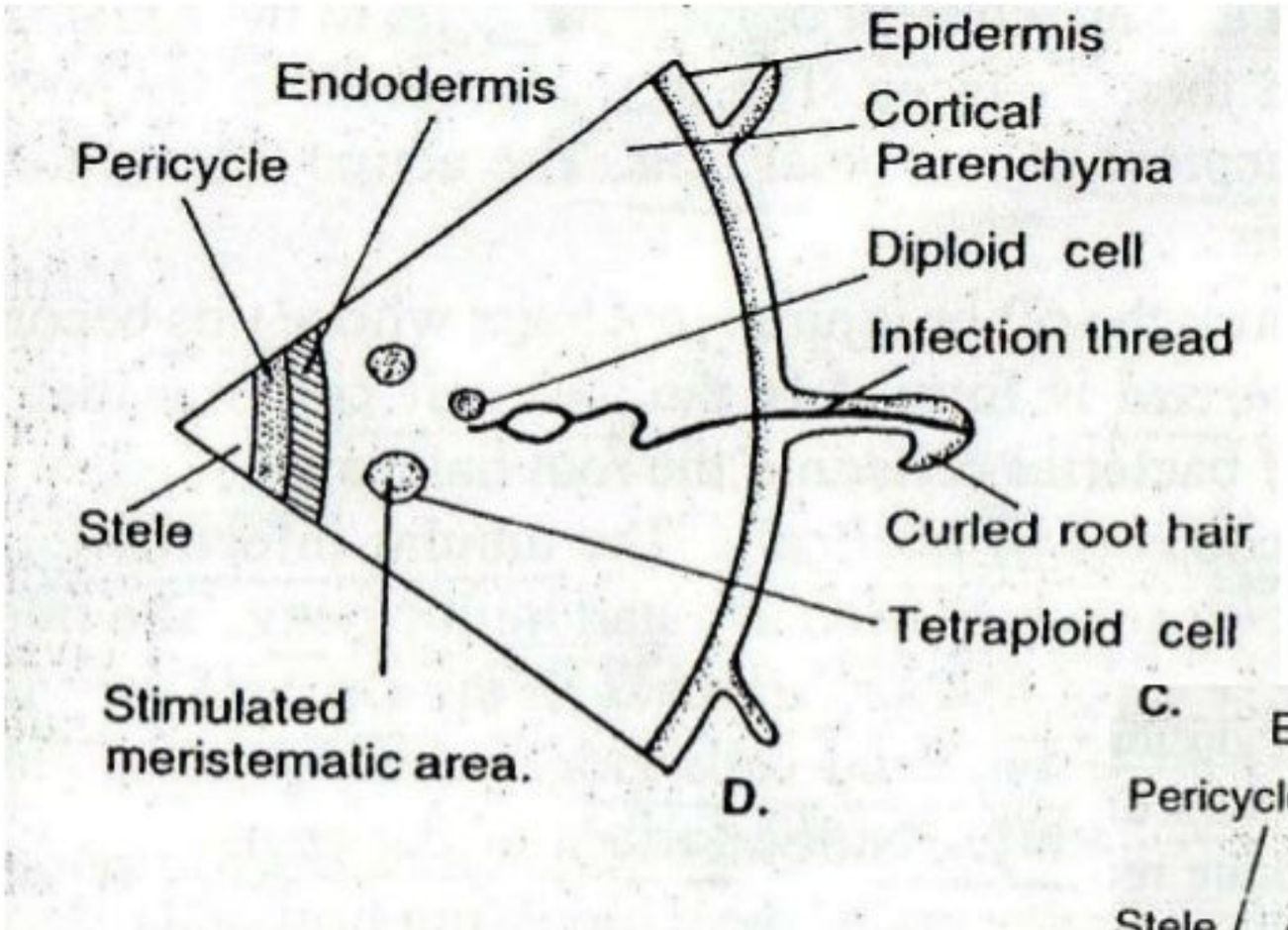
- Root nodules formed due to infection of Rhizobium
- Free living bacteria growing near root of legumes unable to fix nitrogen in free condition
- Roots of the legumes secrete some growth factors helps in fast multiplication of bacteria
- (E.g.) *Pisum sativum* secretes homo serine also carbohydrate containing protein Lectins over their surface

CONTD..

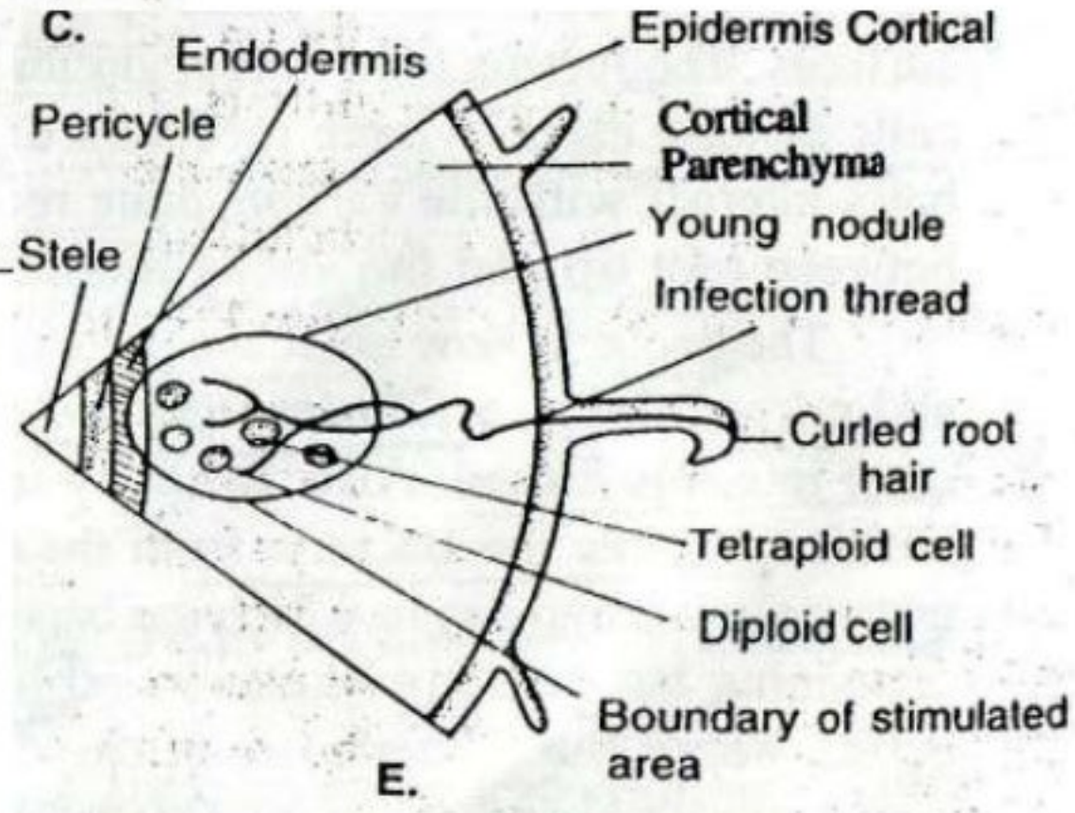
- This helps in recognition and attachment of rhizobial cells
- Rhizobial cells have carbohydrate receptor on their surface
- Lectins interact with the carbohydrate receptor of rhizobial cells
- Occur between root hairs and young root hair
- Bacteria enter the roots through soft infected root hairs
- Tips are deformed and curved
- Tubular infection thread is formed in the root hair cell and bacteria enters into it


Epidermal cell
Soil particles
Root hair Nucleus
Root hair
Cortical Parenchyma cell
Bacteria

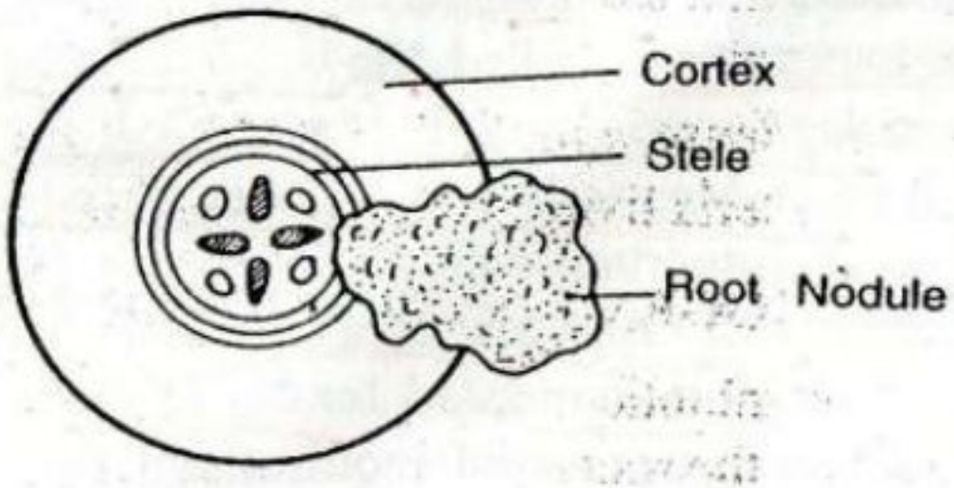

Curved tip
of root hair
Rhizobia
Invagination
Point
Root hair Nucleus
Invagination point
Tubular
Infection thread


Epidermis
Endodermis
Cortical
Parenchyma
Pericycle
Diploid cell
Infection thread
Curled root hair
Stele
Tetraploid cell
Stimulated
meristematic area.

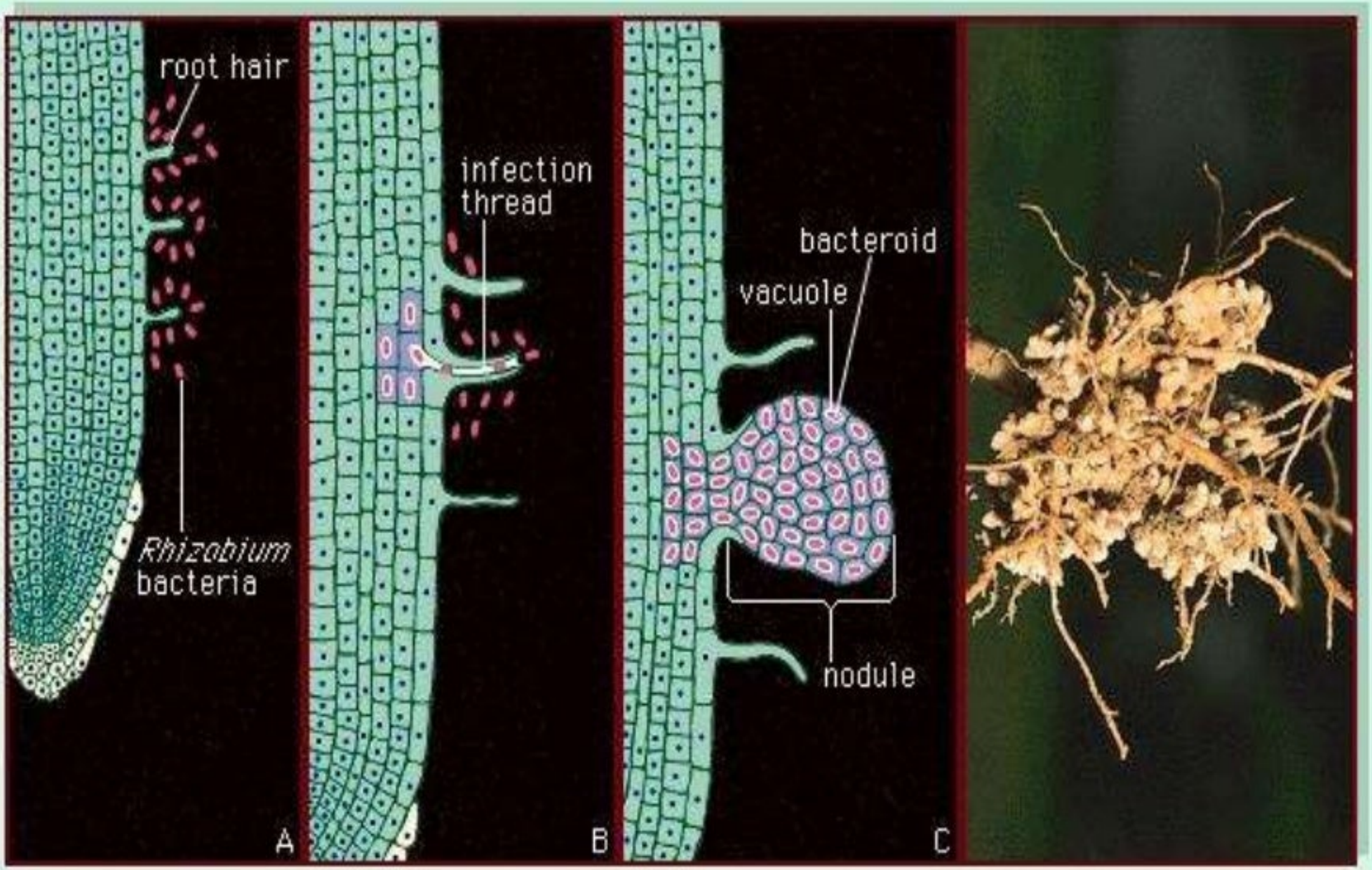
D.


C.
Epidermis Cortical
Endodermis
Pericycle
Cortical
Parenchyma
Young nodule
Stele
Infection thread
Curled root
hair
Tetraploid cell
Diploid cell
Boundary of stimulated
area

E.


Cortex
Stele
Root Nodule

F.



©1996 Encyclopaedia Britannica, Inc.

CONTD..

- After entry, new cell wall is formed
- Tubular infection contains mucopolysaccharides where bacteria embedded and start multiplication
- It grows much and reaches the inner layers of cortex and the bacteria is released
- It induces the cortical cells to multiply which result in the formation of nodule on the surface
- The bacterial cells multiplies and colonize in the multiplying host cells

CONTD..

- After host cells are completely filled, bacterial cells becomes dormant-bacteroids
- Float in leghaemoglobin – reddish pigment in cytoplasm of host cells
  - Efficient O_2 scavenger
  - Maintains steady state of oxygen
  - Stimulates ATP production
- Present studies indicates that leghaemoglobin is not essential
- Nitrogenous compounds synthesized is translocated through vascular tissues
- Groups of rhizobia surrounded by double membrane originated from host cell wall
- Bacteroids lack firm wall (osmotically liable)

BIOCHEMISTRY OF NITROGEN FIXATION

- Basic requirements for Nitrogen fixation
  - Nitrogenase and hydrogenase enzyme
  - Protective mechanism against Oxygen
  - Ferrodoxin
  - Hydrogen releasing system or electron donor (Pyruvic acid or glucose/sucrose)
  - Constant supply of ATP
  - Coenzymes and cofactors TPP, CoA, inorganic phosphate and Mg^{+2}
  - Cobalt and Molybdenum
  - A carbon compound

NITROGENASE ENZYME

- Plays key role
- Active in anaerobic condition
- Made up of two protein subunits
  - Non heme iron protein (Fe-protein or dinitrogen reductase)
  - Iron molybdenum protein (Mo Fe-protein or dinitrogenase)
- Fe protein reacts with ATP and reduces second subunit which ultimately reduces N_2 into ammonia

$$N_2 + 6H^+ + 6e^- \longrightarrow 2NH_3$$

CONTD..

- The reduction of N_2 into NH_3 requires 6 protons and 6 electrons
- 12 mols of ATP required
- One pair of electron requires 4 ATP
- The modified equation

$$N_2 + 8H^+ + 8e^- \longrightarrow 2NH_3 + H_2$$

- Hydrogen produced is catalyzed into protons and electrons by hydrogenase

$$H_2 \xrightleftharpoons{\text{hydrogenase}} 2H^+ + 2e^-$$

PATHWAY OF NITROGEN FIXATION IN ROOT NODULES

- Glucose-6-phosphate acts as a electron donor

Sucrose (synthesized in leaves) → Sucrose (in roots) → Glucose and fructose → Glucose-6-phosphate

- Glucose-6-phosphate is converted to phosphogluconic acid

- NADPH donates electrons to ferrodoxin. Protons released and ferrodoxin is reduced
- Reduced ferrodoxin acts as electron carrier. Donate electron to Fe-protein to reduce it. Electrons released from ferrodoxin thus oxidized

CONTD..

- Reduced Fe-protein combines with ATP in the presence of Mg^{+2}
- Second sub unit is activated and reduced
- It donates electrons to N_2 to NH_3
- Enzyme set free after complete reduction of N_2 to NH_3