Plant Nutrition in Horticulture

Bahram Baninasab

Department of Horticulture
Isfahan University of Technology

Nitrogen

Nitrification

NH_3 ---------------→ NO_2^- ---------------→ NO_3^- ↓

Nitrosmonase + O_2 | Nitrobacter | Leaching

Denitrification

NO_3^- ---------------→ N_2↑ , N_2O↑

Heterotrophic bacteria
(*Paracocus denitrificans*)
- O_2

Urease

$(NH_2)_2CO$ -----------→ $CO_2 + 2NH_3$

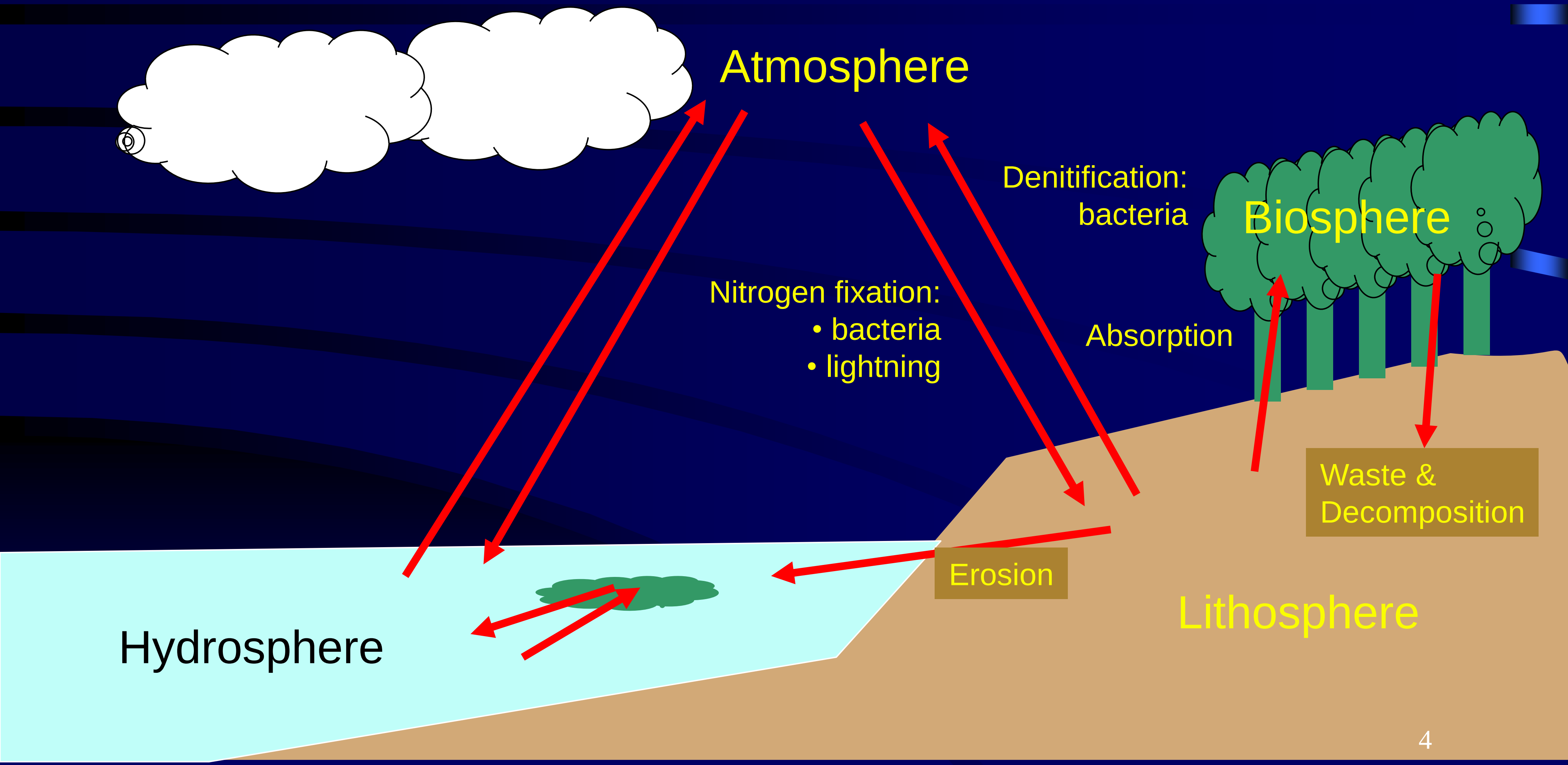
Source of Nitrogen

Atmosphere (78 % of atmospheric gases)
Lithosphere
Hydrosphere
Biosphere

Bacteria → Prokaryote

$$N_2 \xrightarrow[\text{Nitrogenase}]{\text{---------------}} NH_3$$

Nitrogen Cycle



Nitrogen Fixation

Three processes are responsible for most of the nitrogen fixation:

- Atmospheric fixation
- Industrial fixation
- Biological fixation

Atmospheric Nitrogen Fixation

A relatively small amount of nitrogen fixation occurs during lightning

$$N_2 + O_2 \xrightarrow{\text{lightning}} 2\,NO$$

$$2\,NO + O_2 \longrightarrow 2NO_2$$

$$2\,NO_2 + H_2O \longrightarrow HNO_3 + HNO_2$$

The enormous amounts of energy released by lightning break nitrogen molecules apart, allowing their atoms to combine with oxygen and form nitrogen oxides

These *oxides* dissolve in *rain* and form *nitrates* that are carried to the earth's surface

Nitrates formed during thunderstorms account for around 5-8% of total nitrogen fixation (30 Kg NO_3^- $Hec.^{-1}$ $Year^{-1}$)

Industrial Nitrogen Fixation (Haber-Bosch)

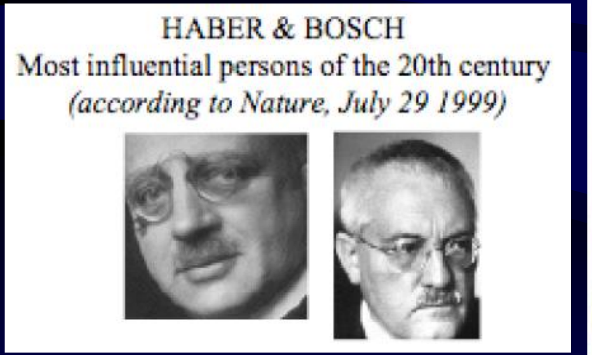
High pressure (20-80 MP)

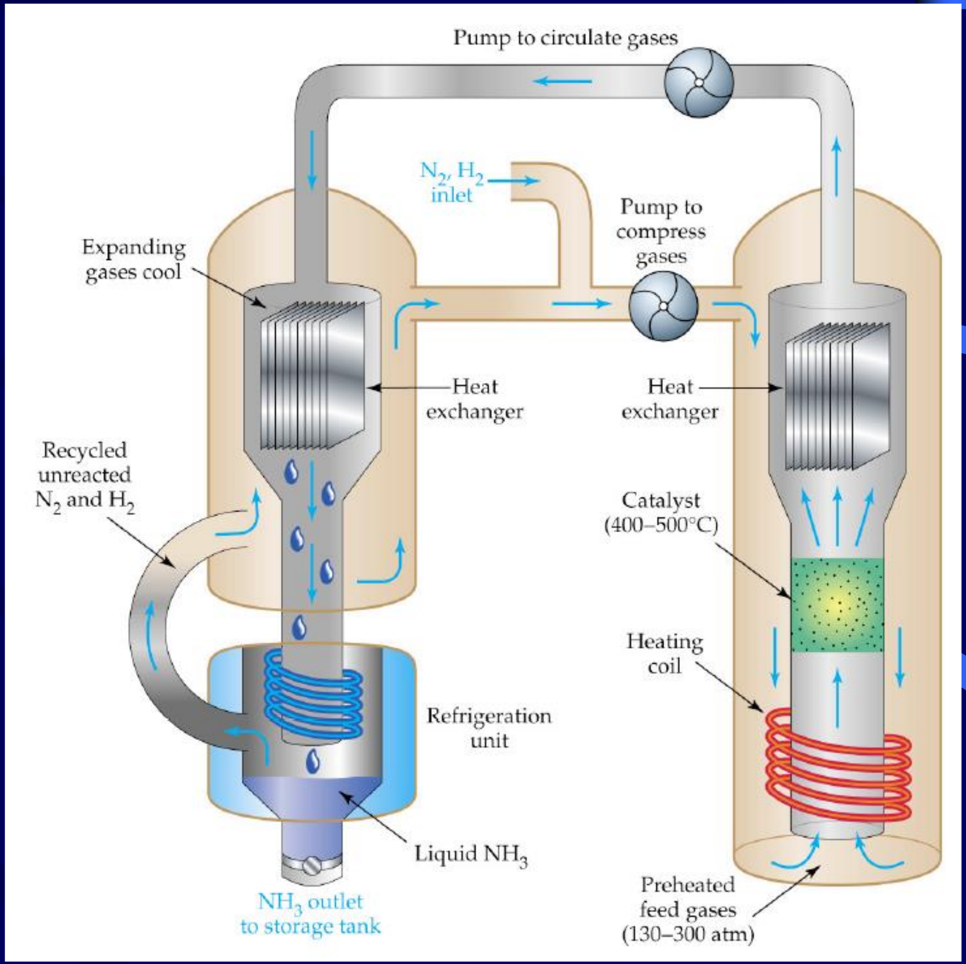
$$N_2 + 3H_2 \text{---------------------}\rightarrow 2NH_3$$

High temperature (300-600 °C)

High Cost, Fossil energy

1 Kg $NH_3 \approx$ 1.5 Kg fossil energy





Biological Nitrogen Fixation

Free livening and Symbiosis

- Scientist estimate that biological fixation globally adds approximately 140 million tons of nitrogen to ecosystems every year.

Advantages

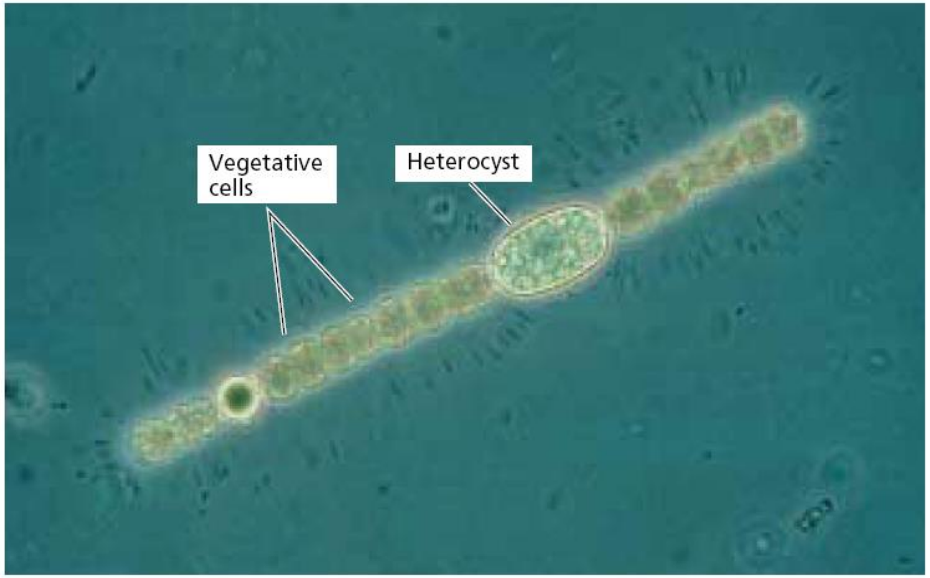
- Sun energy
- **T**he first stable product of the process is ammonia, this is quickly incorporated into protein and other organic nitrogen compounds
- Transport cost

Biological Nitrogen Fixation

- Symbiotic N Fixation
- Free-Living N Fixation

- Blue-Green Algae (Cyanobacteria) : *Anabaena cylindrica*

FIGURE 12.9 A heterocyst in a filament of the nitrogen-fixing cyanobacterium *Anabaena*. The thick-walled heterocysts, interspaced among vegetative cells, have an anaerobic inner environment that allows cyanobacteria to fix nitrogen in aerobic conditions. (© Paul W. Johnson/ Biological Photo Service.)



Symbiotic N Fixation

Bacteria

- *Frankia*

- *Rhizobium*

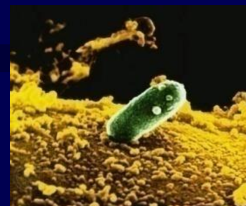


TABLE 12.2
Examples of organisms that can carry out nitrogen fixation

Symbiotic nitrogen fixation	
Host plant	**N-fixing symbionts**
Leguminous: legumes, *Parasponia*	*Azorhizobium, Bradyrhizobium, Photorhizobium, Rhizobium, Sinorhizobium*
Actinorhizal: alder (tree), *Ceanothus* (shrub), *Casuarina* (tree), *Datisca* (shrub)	*Frankia*
Gunnera	*Nostoc*
Azolla (water fern)	*Anabaena*
Sugarcane	*Acetobacter*
Free-living nitrogen fixation	
Type	**N-fixing genera**
Cyanobacteria (blue-green algae)	*Anabaena, Calothrix, Nostoc*
Other bacteria	
Aerobic	*Azospirillum, Azotobacter, Beijerinckia, Derxia*
Facultative	*Bacillus, Klebsiella*
Anaerobic	
Nonphotosynthetic	*Clostridium, Methanococcus* (archaebacterium)
Photosynthetic	*Chromatium, Rhodospirillum*

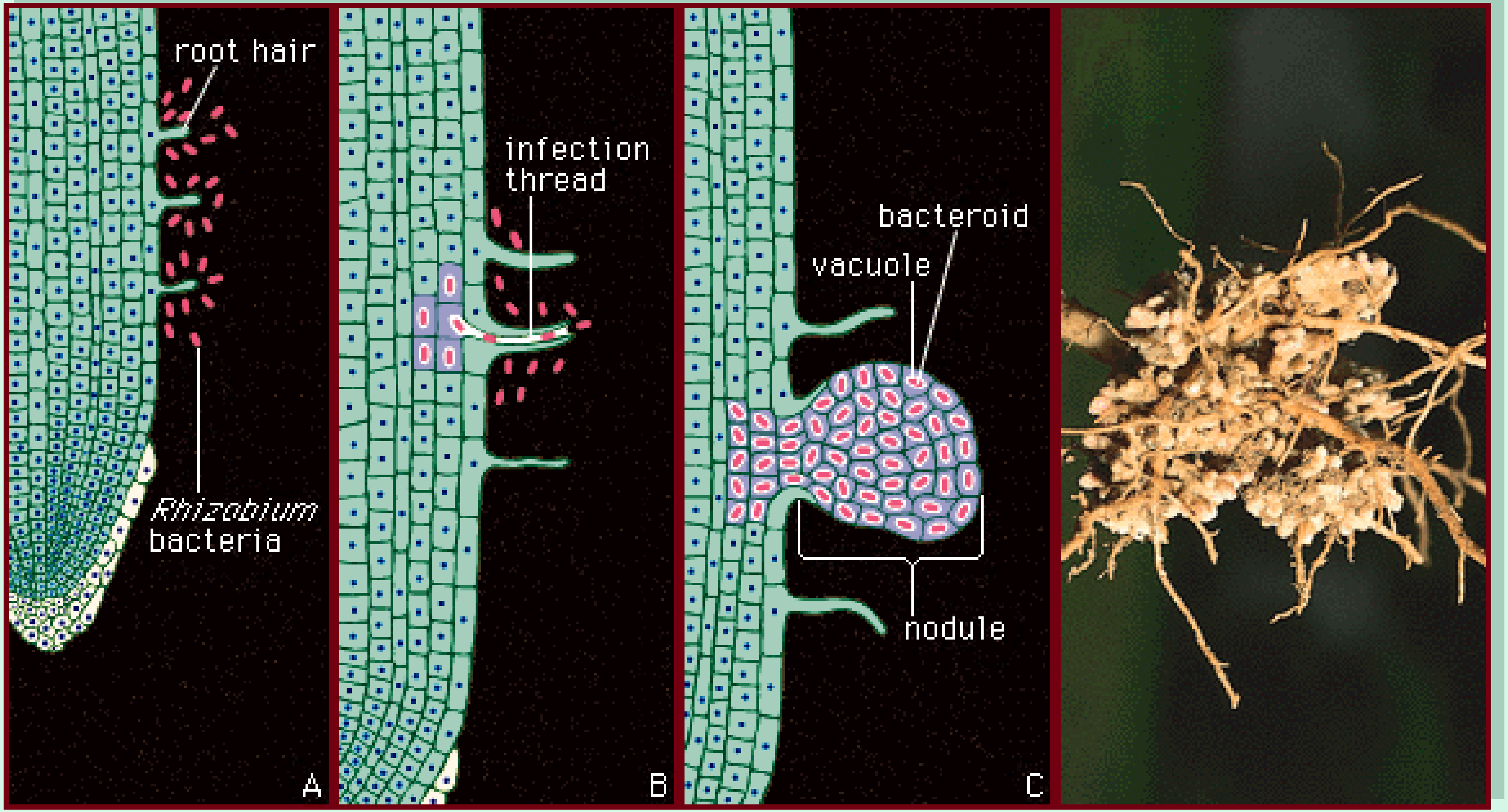
TABLE 12.3
Associations between host plants and rhizobia

Plant host	Rhizobial symbiont
Parasponia (a nonlegume, formerly called *Trema*)	*Bradyrhizobium* spp.
Soybean (*Glycine max*)	*Bradyrhizobium japonicum* (slow-growing type); *Sinorhizobium fredii* (fast-growing type)
Alfalfa (*Medicago sativa*)	*Sinorhizobium meliloti*
Sesbania (aquatic)	*Azorhizobium* (forms both root and stem nodules; the stems have adventitious roots)
Bean (*Phaseolus*)	*Rhizobium leguminosarum* bv. *phaseoli*; *Rhizobium tropicii*; *Rhizobium etli*
Clover (*Trifolium*)	*Rhizobium leguminosarum* bv. *trifolii*
Pea (*Pisum sativum*)	*Rhizobium leguminosarum* bv. *viciae*
Aeschenomene (aquatic)	*Photorhizobium* (photosynthetically active rhizobia that form stem nodules, probably associated with adventitious roots)

Estimated Average Rates of Biological N_2 Fixation

Organism or system	N_2 fixed (kg ha^{-1} y^{-1})
Free-living microorganisms	
Cyanobacteria	25
Azotobacter	0.3
Clostridium pasteurianum	0.1-0.5
Leguminous plant symbioses with rhizobia	
Grain legumes	50-100
Pasture legumes (*Trifolium, Medicago, Lupinus*)	100-600
Plant symbioses with *Frankia*	
Alnus	40-300
Hippophae	1-150
Ceanothus	1-50
Coriaria	50-150
Casuarina	50

Rhizobium Root Nodules



Role of Root Exudates

General

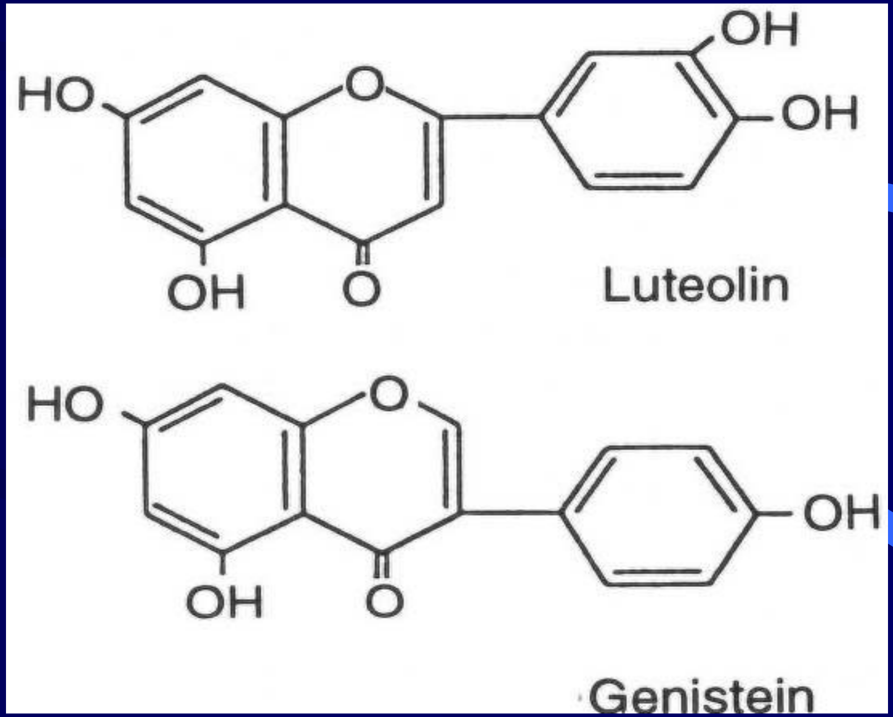
- Amino acids, Sugars, Flavones

Specific

- Flavones

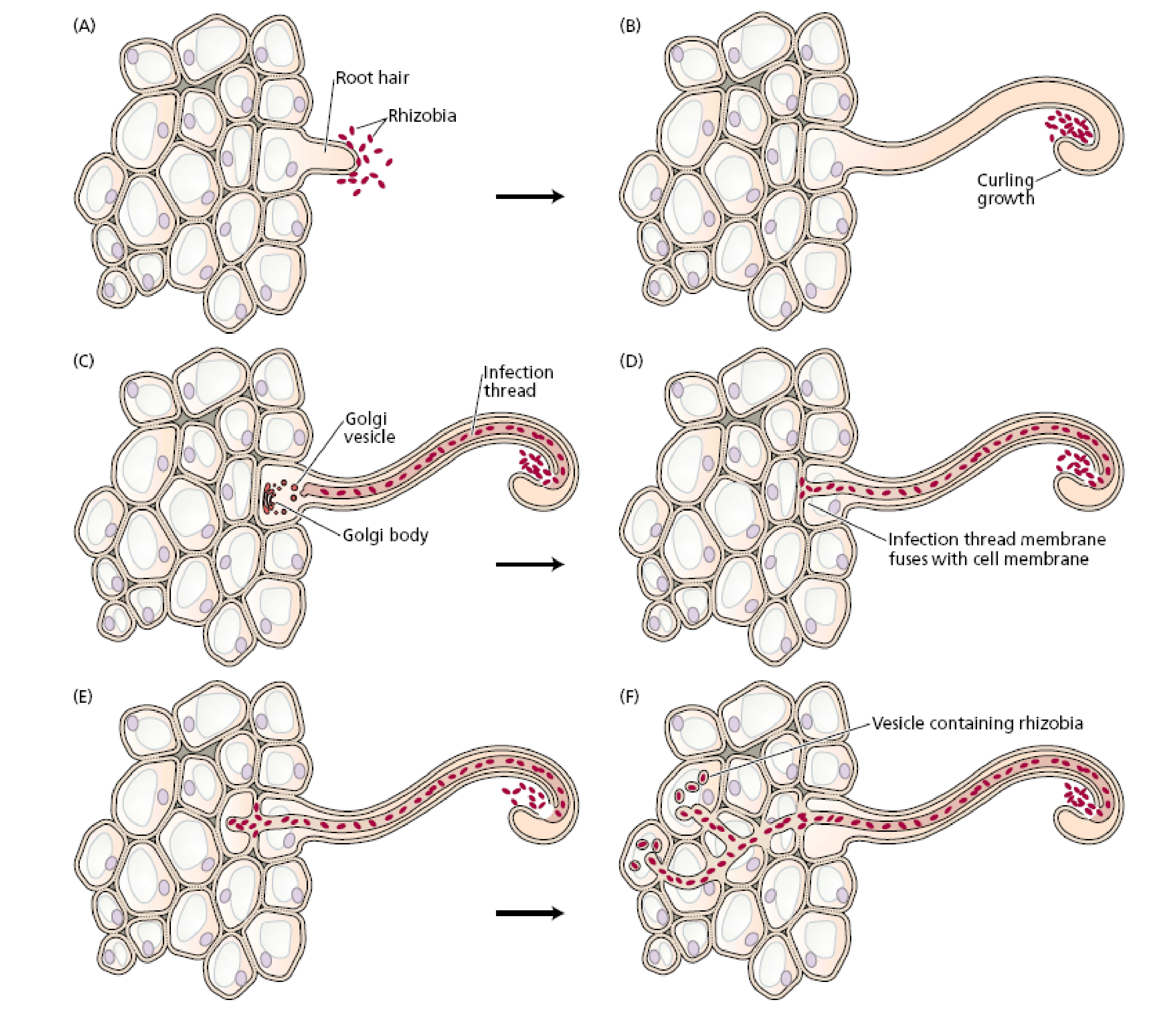
  Luteolin (Melilotus)

  Genistein (Phaseolus)

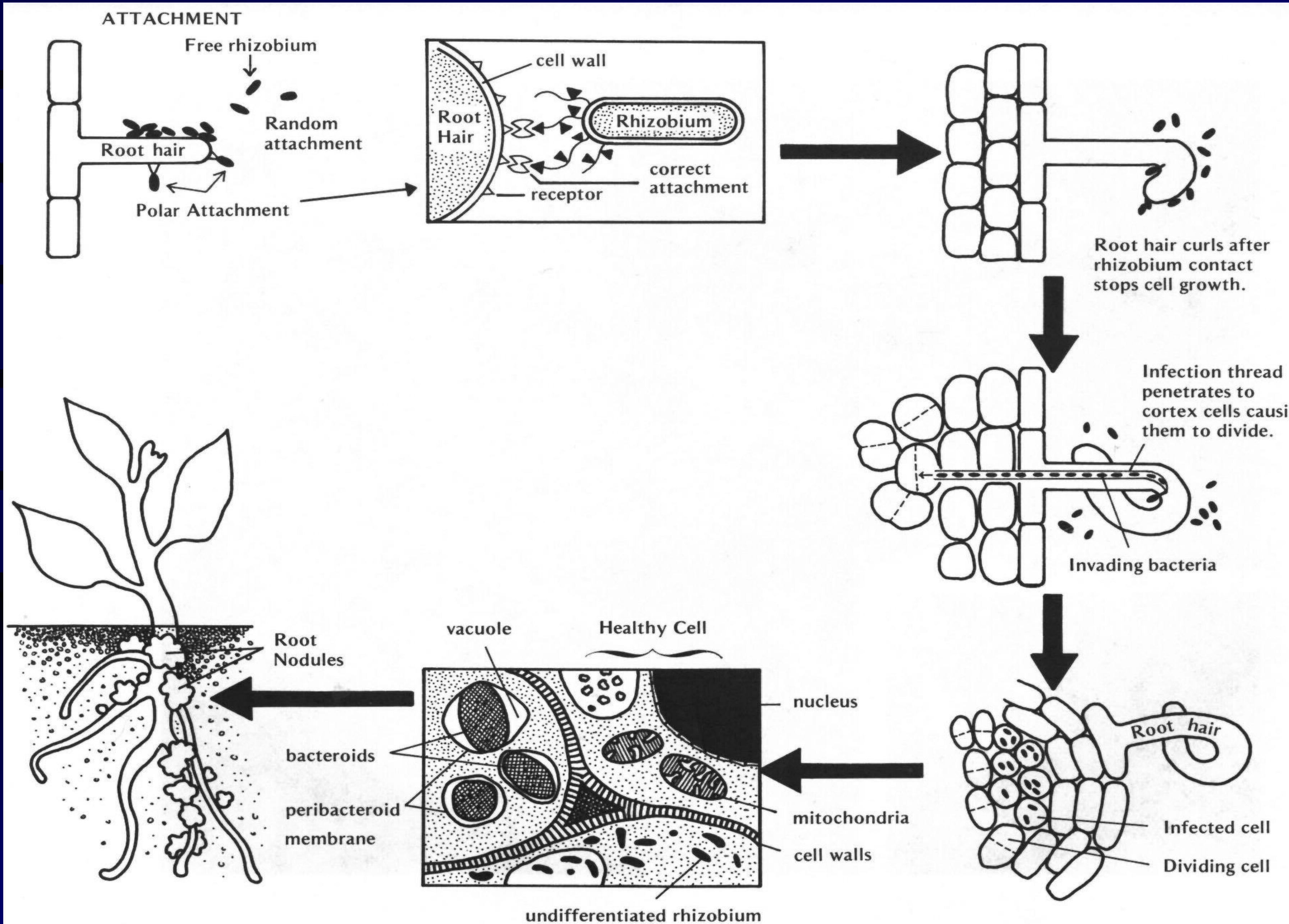


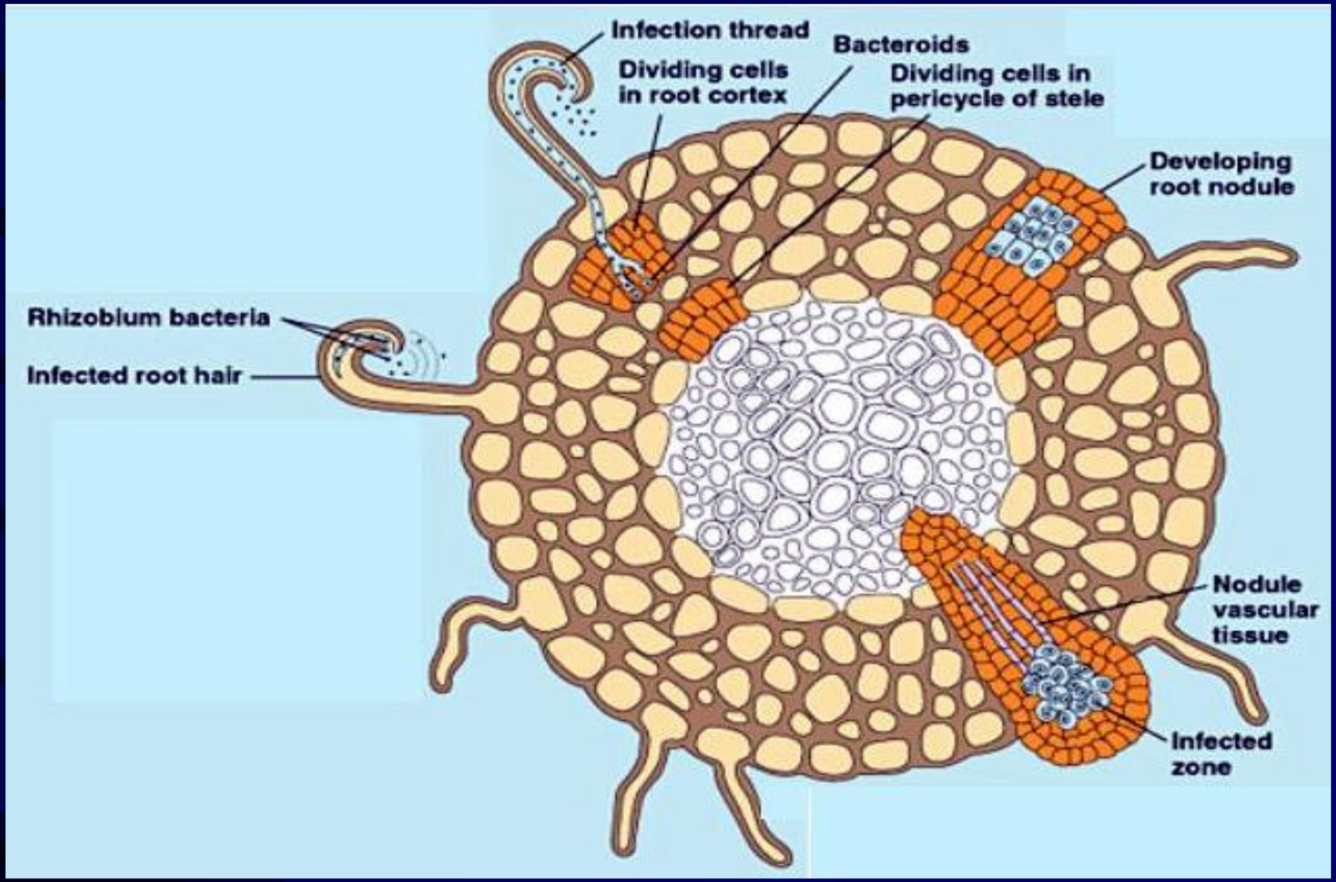
- Vary by plant species
- Responsiveness varies by rhizobia species

The infection process during nodule organogenesis.
(A) Rhizobia bind to an emerging root hair in response to chemical attractants sent by the plant. (B) In response to factors produced by the bacteria, the root hair exhibits abnormal curling growth, and rhizobia cells proliferate within the coils. (C) Localized degradation of the root hair wall leads to infection and formation of the infection thread from Golgi secretory vesicles of root cells. (D) The infection thread reaches the end of the cell, and its membrane fuses with the plasma membrane of the root hair cell. (E) Rhizobia are released into the apoplast and penetrate the compound middle lamella to the subepidermal cell plasma membrane, leading to the initiation of a new infection thread, which forms an open channel with the first. (F) The infection thread extends and branches until it reaches target cells, where vesicles composed of plant membrane that enclose bacterial cells are released into the cytosol.



Nodulation in Legumes



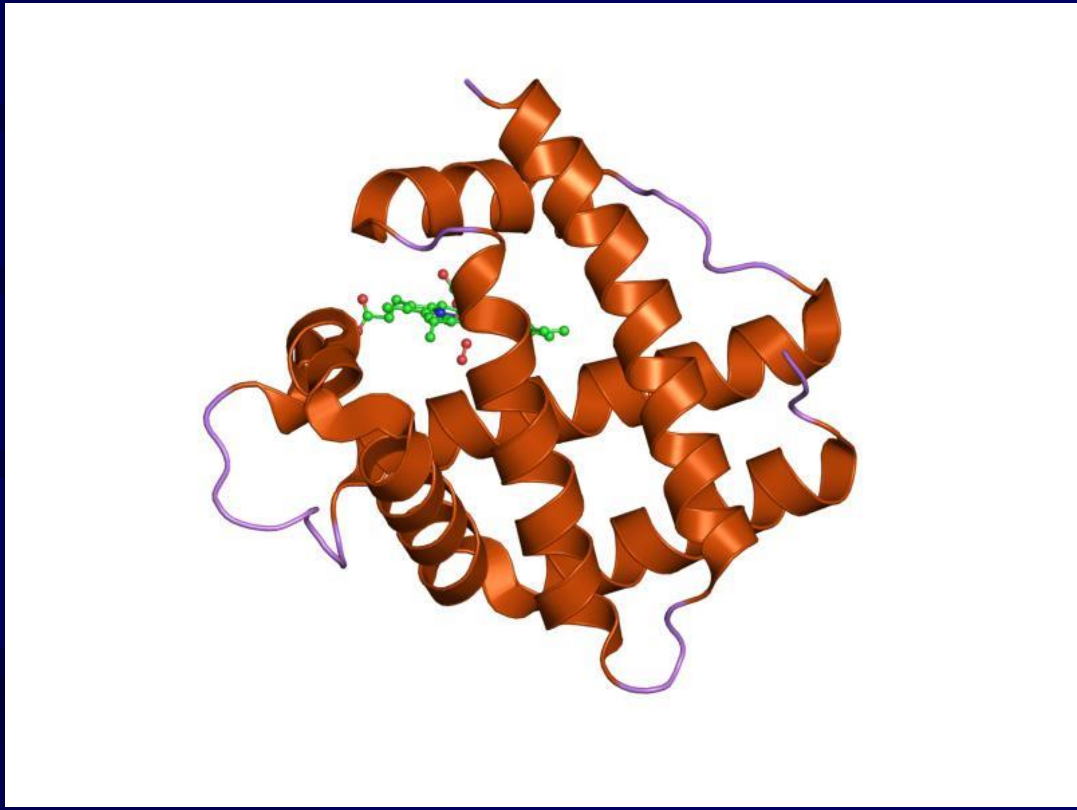


Soya bean infection
with *Rhizobium*

Leghemoglubin

Heam (Bacteria)

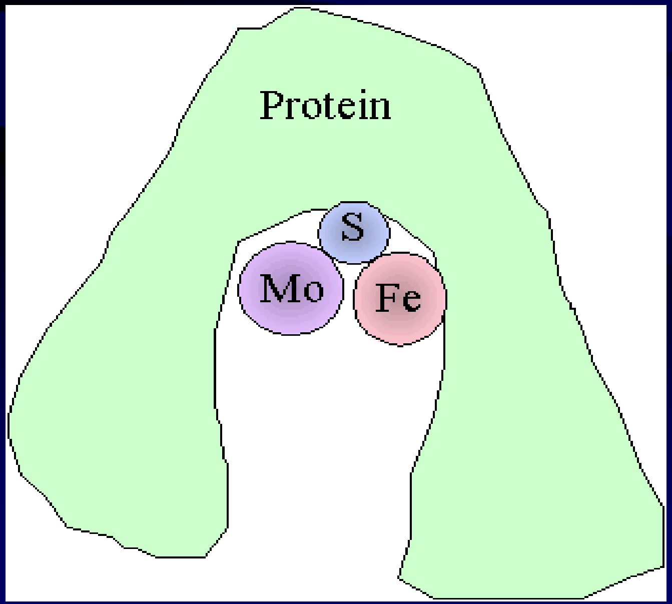
Apoprotein   (Host)



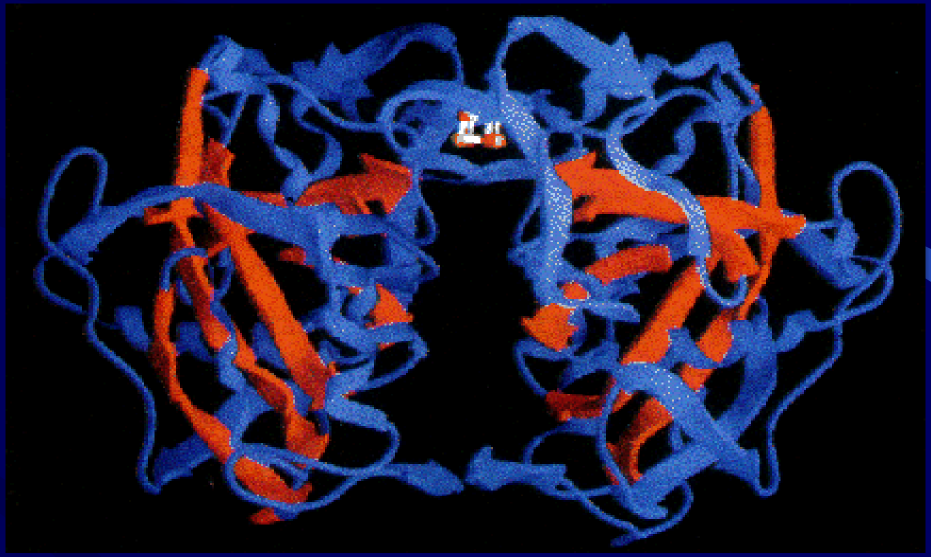
Nitrogen-Fixing Bacteria in Root Nodules

Nitrogen Fixation

- All nitrogen fixing bacteria use highly conserved enzyme complex called **Nitrogenase**
- Nitrogenase is composed of of two subunits: an iron-sulfur protein and a molybdenum-iron-sulfur protein



Nitrogenase



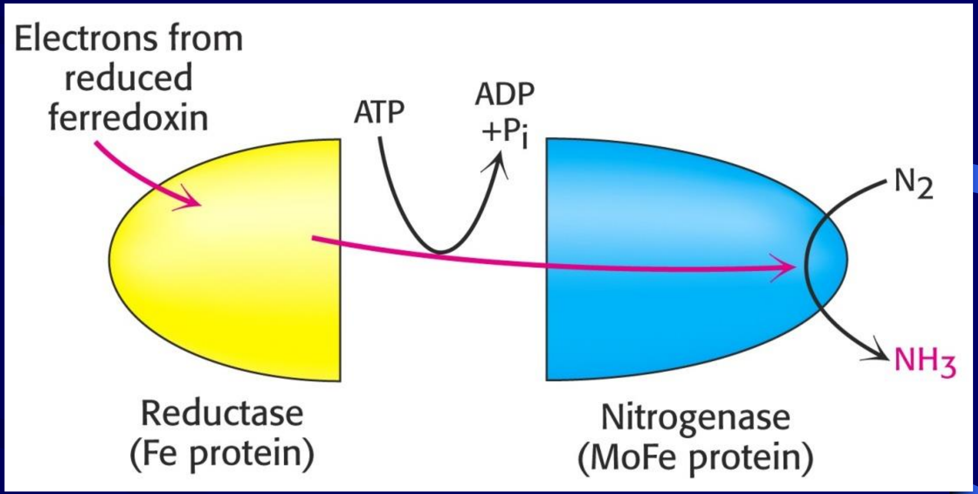
Nitrogenase Structure

Fe-S-Protein

Molecular weight 6000 Dalton

4 Fe, 4 S

Function: Electron carrier



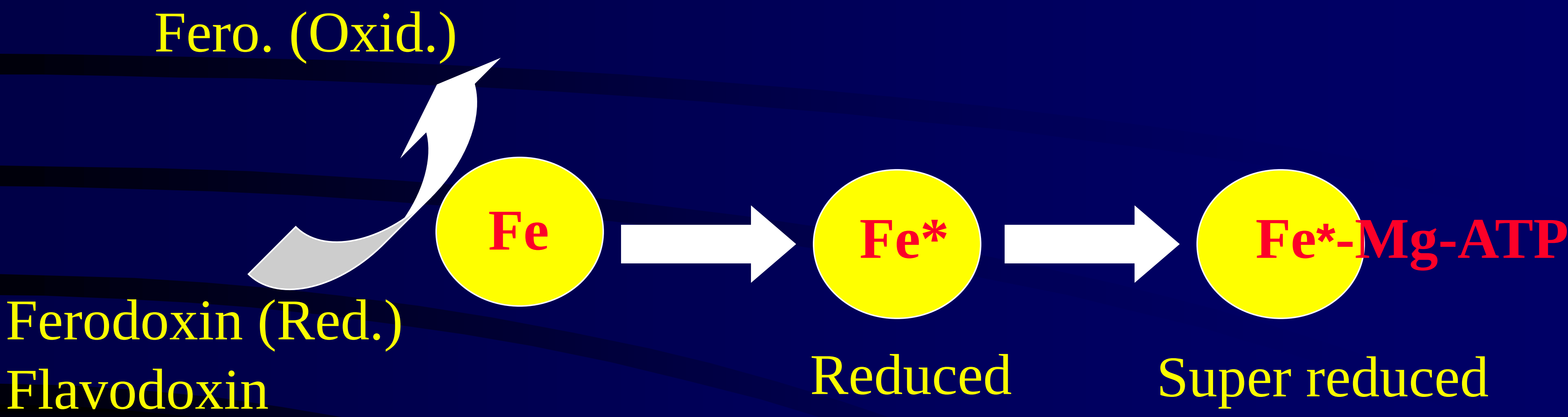
Mo-Fe-S-Protein

Molecular weight 20000 Dalton

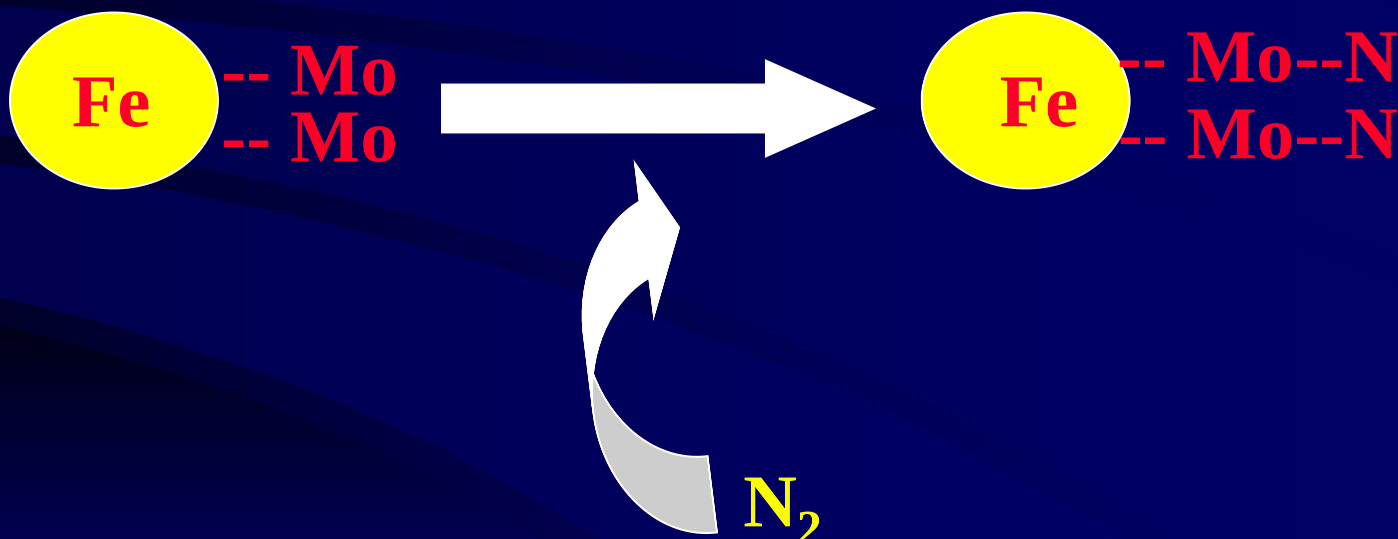
1-2 Mo, 12-32 Fe, 16-24 S

Function: $N_2 \rightarrow 2NH_3$

Fe-S-Protein


Fero. (Oxid.)
Fe
Fe*
Fe*-Mg-ATP
Ferodoxin (Red.)
Flavodoxin
Reduced
Super reduced

Mo-Fe-S-Protein



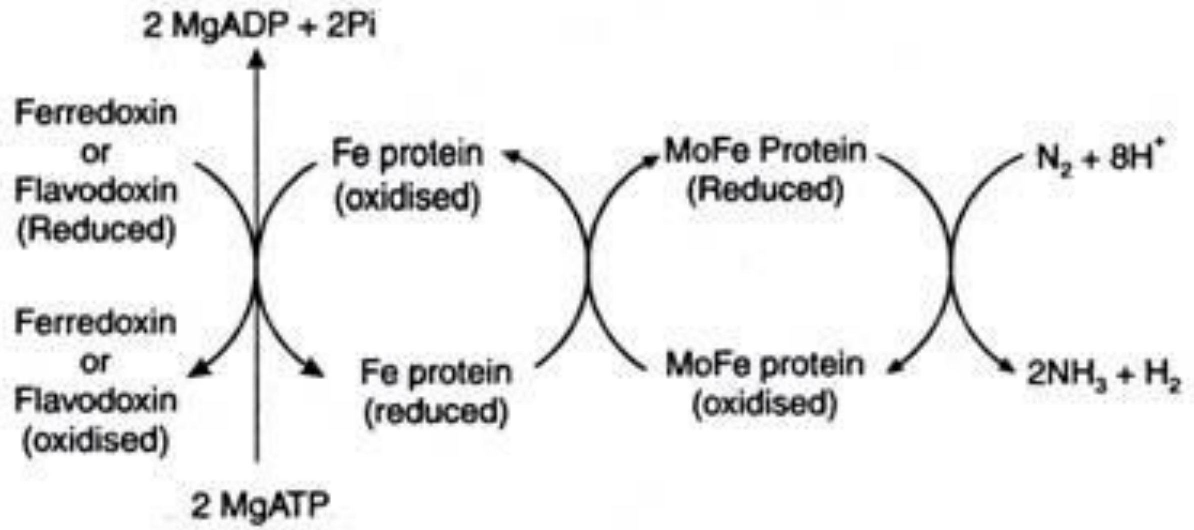
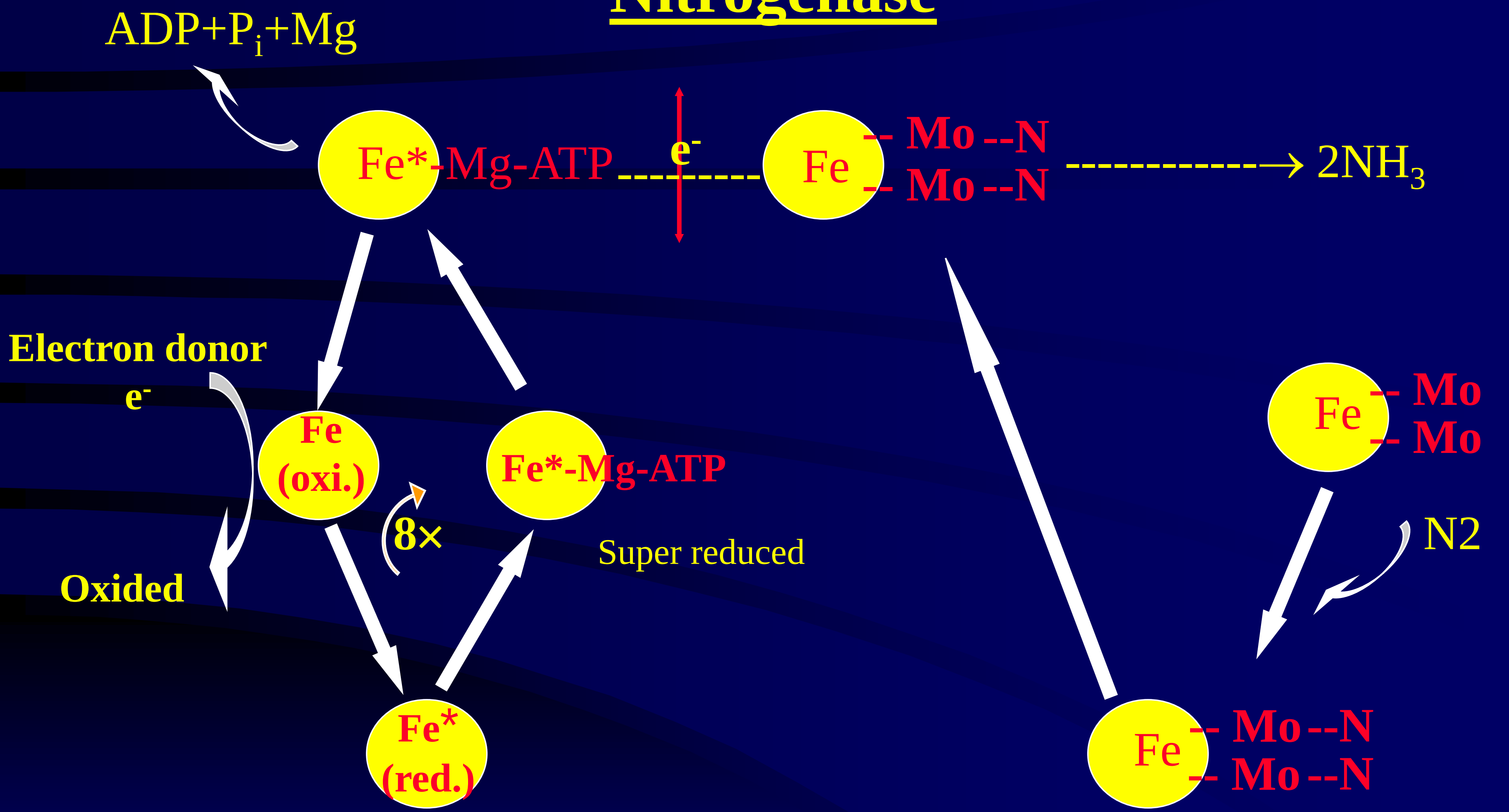


Fig. 9.4. Recent view of mechanism of biological nirogen fixation.

Nitrogenase



$$N_2 + 8H^+ + 8e^- + 16\ MgATP \rightarrow 2NH_3 + H_2 + 16MgADP$$

Reactions Catalyzed By Nitrogenase

$N_2 + 8H^+ + 8e^- + 16\ MgATP \rightarrow 2NH_3 + H_2 + 16MgADP$

Energy efficiency evaluation:

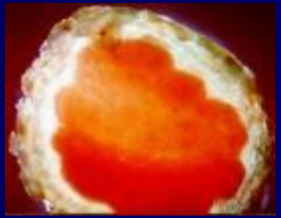
The Mo-Fe protein can reduce many substrates
Although under natural conditions the Mo-Fe only reacts with N_2 and H^+.

Substrate		**Product**
N_2	→	$2NH_3$
$2H^+$	→	H_2
C_2H_2	→	C_2H_4

Factors affecting Nitogenase activity

- ATP/ADP = 10
- Nitrogenase activity is inactivated by oxygen
- ATP requirement for nitrogen fixation
- Oxygen requirement for ATP synthesis

1. The site of nitrogen fixation in Blue-Green algae separated from oxygen produced site



Isolated LegHemoglobin

2. LegHemoglobin keeps $[O_2]$ low

Turnover Number of Nitrogenase: 3

Per Second:

$3N_2 \rightarrow 6NH_3$

72 ATP

Final Product

$N_2 + 8H^+ + 8e^- + 16\ MgATP \rightarrow 2NH_3 + H_2 + 16MgADP$

$$NH_3 \rightleftharpoons NH_4^+$$

Pk=9.25

Ammonium Assimilation

Ammonium is toxic in high concentrations, therefore it must be maintained at low levels. Therefore there are several mechanisms by which ammonium is trapped into organic molecules.

Two Systems

- Low Efficiency Sys. (Without ATP)
- High Efficiency Sys. (With ATP)

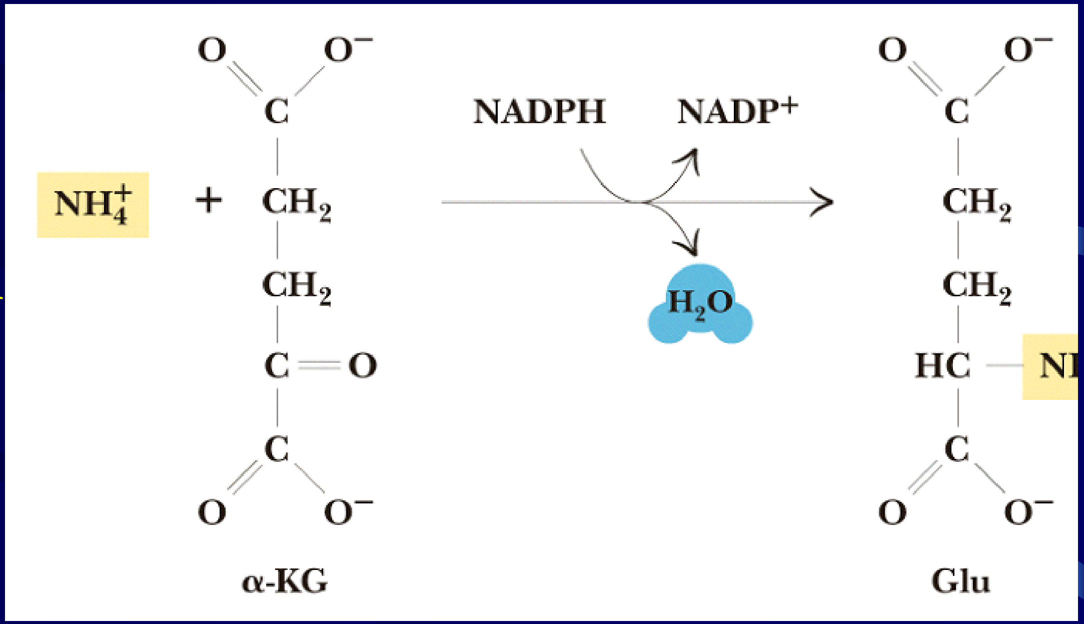
Low efficiency System

Enzyme:

- Glutamate Dehydrogenase

- In plant systems enzyme is localized within the mitochondria of root and leaf cell

- Glutamate dehydrogenase reductive amination of
- α-ketogluturate to glutamate



α-ketogluturate Glutamic Acid

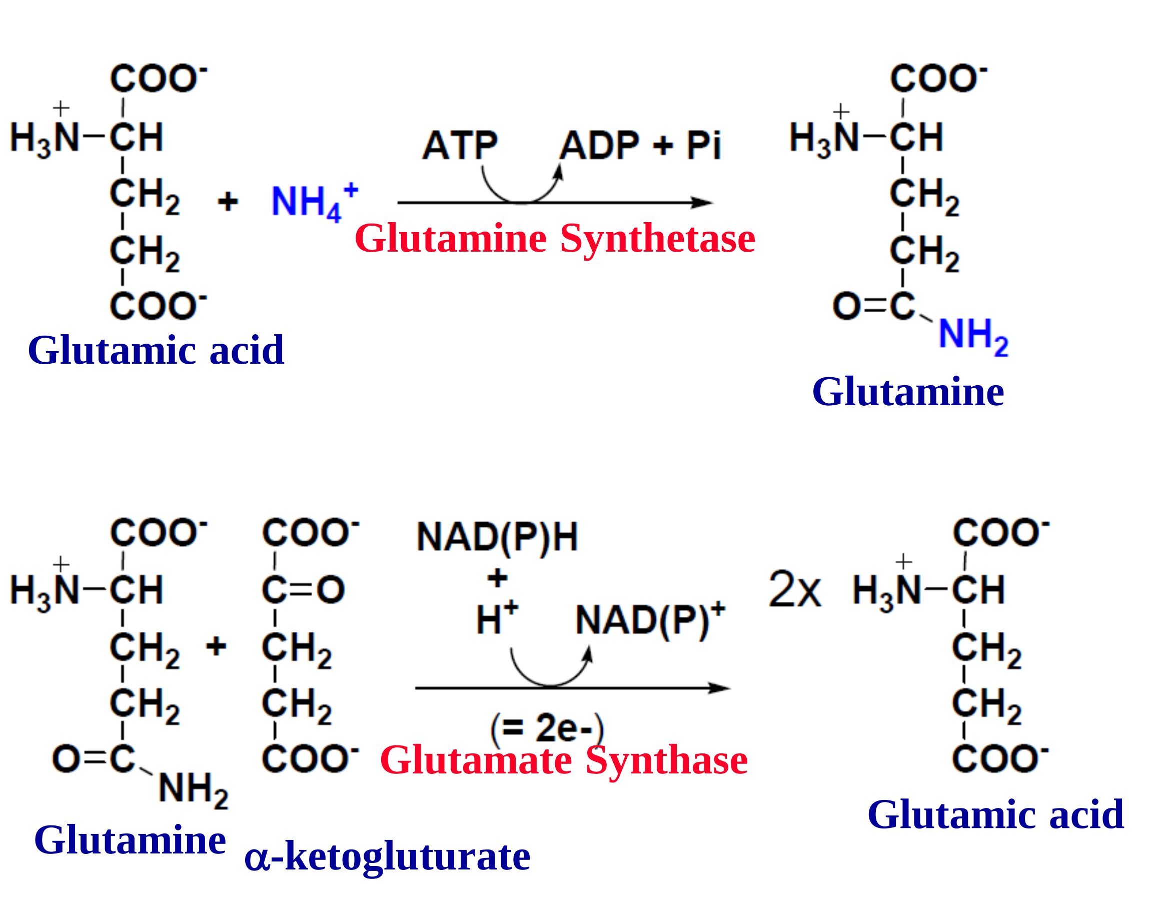
Km = 14 mM

High efficiency and with ATP system

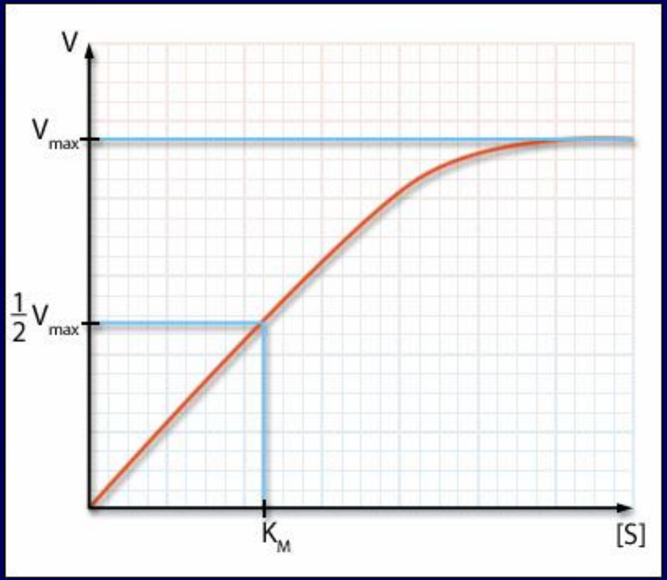
Enzymes:

- Glutamine Synthetase (Km=0.02 mM)
- Glutamate Synthase (Km=0.15mM)

In plant systems enzyme is localized within the root (cytoplasm and plastid) and leaf chloroplast



Enzyme Kinetic Properties

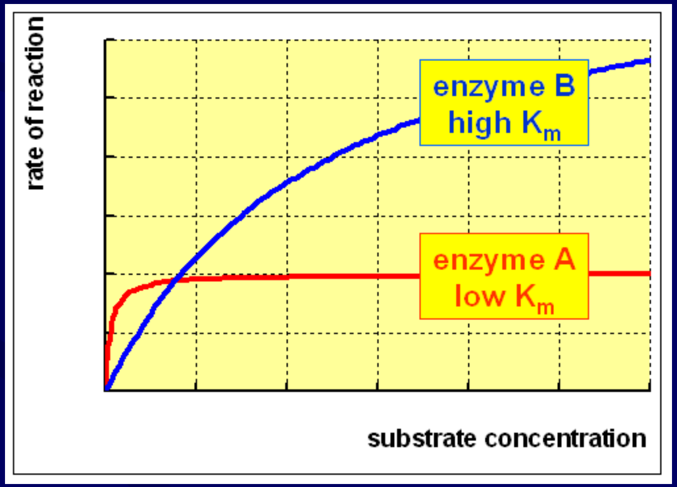


Km is a measure of the affinity that an enzyme has for a given substrate

A high Km = a low affinity

A low Km = a high affinity

Km = [S] at 1/2 Vmax



Glutamin	N/C = 2/5	Gramineae
Asparagin	N/C = 2/4	Lupin
Arginin	N/C = 4/6	Rose
Allantoin	N/C = 4/4	Peanut

Nitrate Assimilation and Reduction

Plants can store high levels of nitrate or translocate it via the xylem without any effect

All plants can take up N in the form of NH_4^+ and nitrate (NO_3^-)

Nitrate must be reduced to NH_4^+ before it can be incorporated into amino acids, proteins and nucleic acids.

$NO_3^- + 8e^- + 8H^+$ ---------> $NH_4^+ + 2H_2O + OH^-$

Two enzymes reduce NO_3 -----> NH_3

A. Nitrate Reductase (Cytoplasm root and shoot cell)

$NO_3^- + NADPH_2 + 2H^+$ ----------> $NO_2^- + NADP + H_2O$

B. Nitrite Reductase

Leaf plastid: $NO_2^- + 6\ Fred + 6H^+$----> $NH_4^+ + 2H_2O$

Root proplastide: $NO_2^- + 3NADPH + 5H^+$-----> $NH_4^+ + 2H_2O + NADP^+$

Nitrate Assimilation and Reduction

- **Absorption: Active , needs energy**
- **Sites of assimilation:**

  Root—10-30%

  Shoot—70-90%

  Vacuole—storage when it is more than needed

- **Process**

$$\overset{+5}{NO_3^-} \xrightarrow[\text{NR}]{2e^-} \overset{+3}{NO_2^-} \xrightarrow[\text{NiR}]{6e^-} \overset{-3}{NH_3}$$

NR: Nitrate reductase; NiR: Nitrite reductase

$$NO_3^- + 8H^+ + 8e^- \longrightarrow NH_3 + 2H_2O + OH^- \quad (\uparrow pH)$$

Nitrate reductase: Cytoplasm root and shoot cell

Nitrite reductase: Chloroplast of leaf cell, Proplastid of root cell

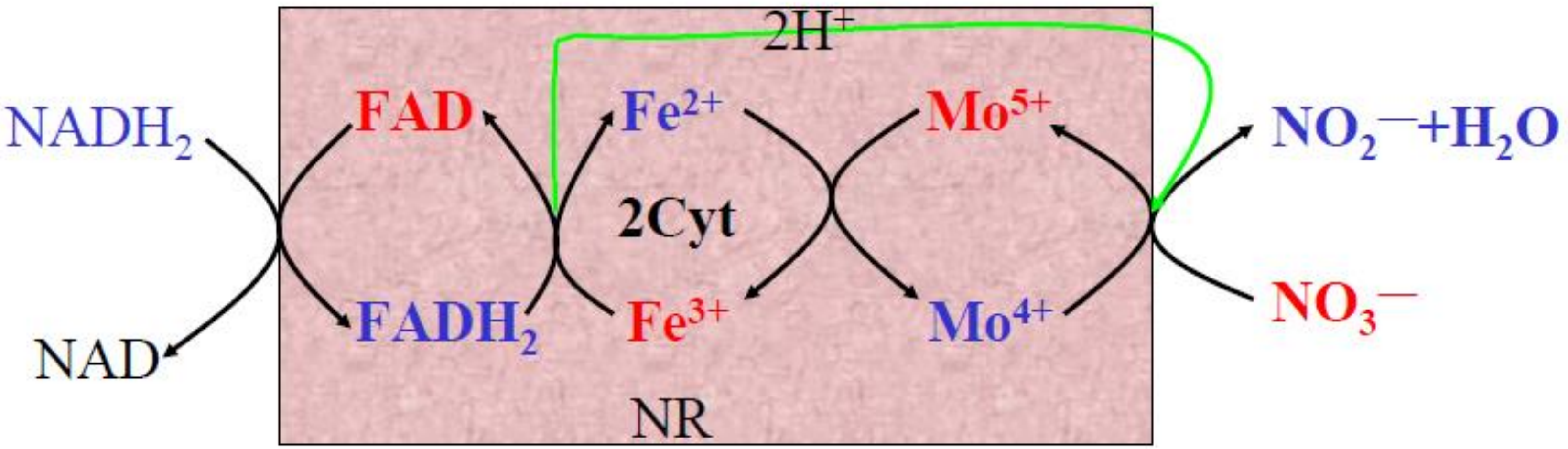
- In many plants, when the roots receive small amounts of nitrate, this nitrate is reduced primarily in the roots
- As nitrate supply increases, a greater proportion of the absorbed nitrate is translocated to the shoot and assimilated there

- In plants, when the roots receive only ammonium , the nitrate reductase content is low
- With change nitrogen source from ammonium to nitrate, the nitrate reductase content increased
- Molybdenum defficiency reduced nitrate reductase activity and disrupted nitrogen metbolism in plant

Nitrate Reductase

Reduction of nitrate to nitrite

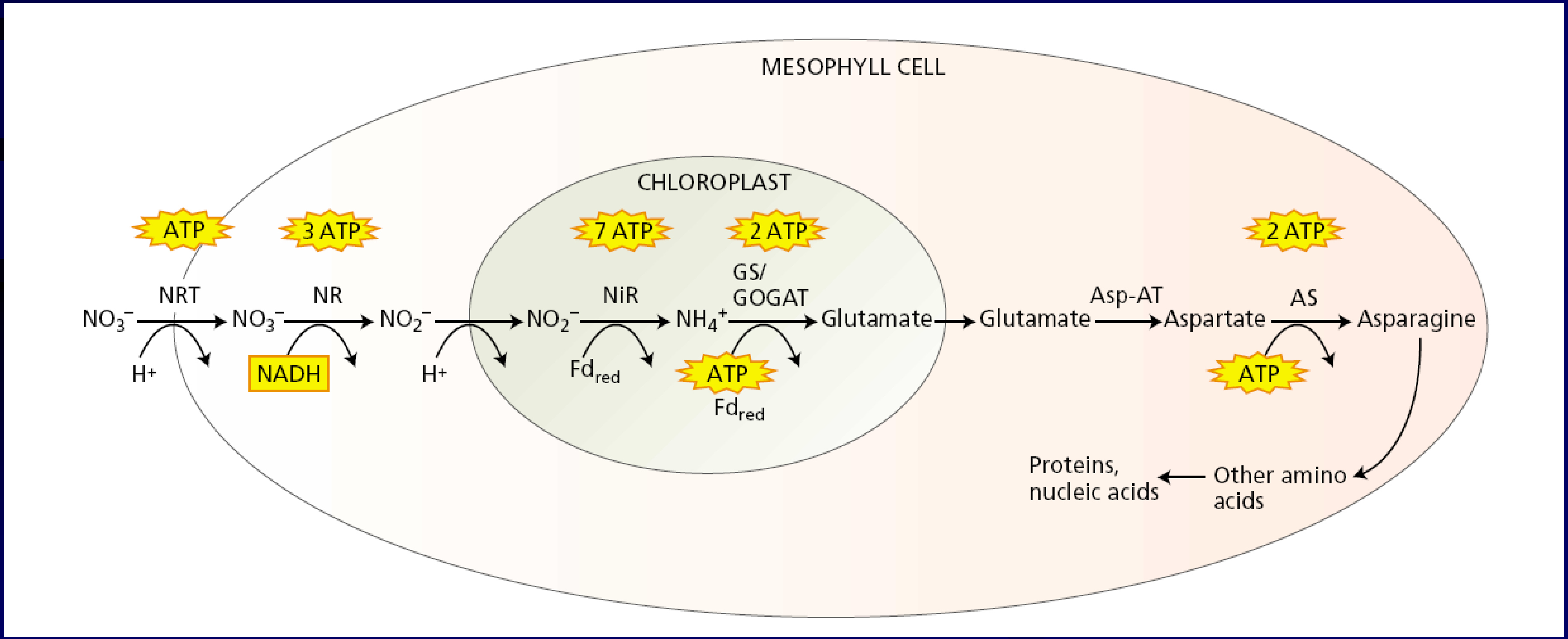
Occur in cytosol



Nitrite Reductase

Leaf plastid: $NO_2^- + 6\ Fred + 6H^+ \text{---->} NH_4^+ + 2H_2O$

Root proplastide: $NO_2^- + 3NADPH + 5H^+ \text{---->} NH4^+ + 2H_2O + NADP^+$



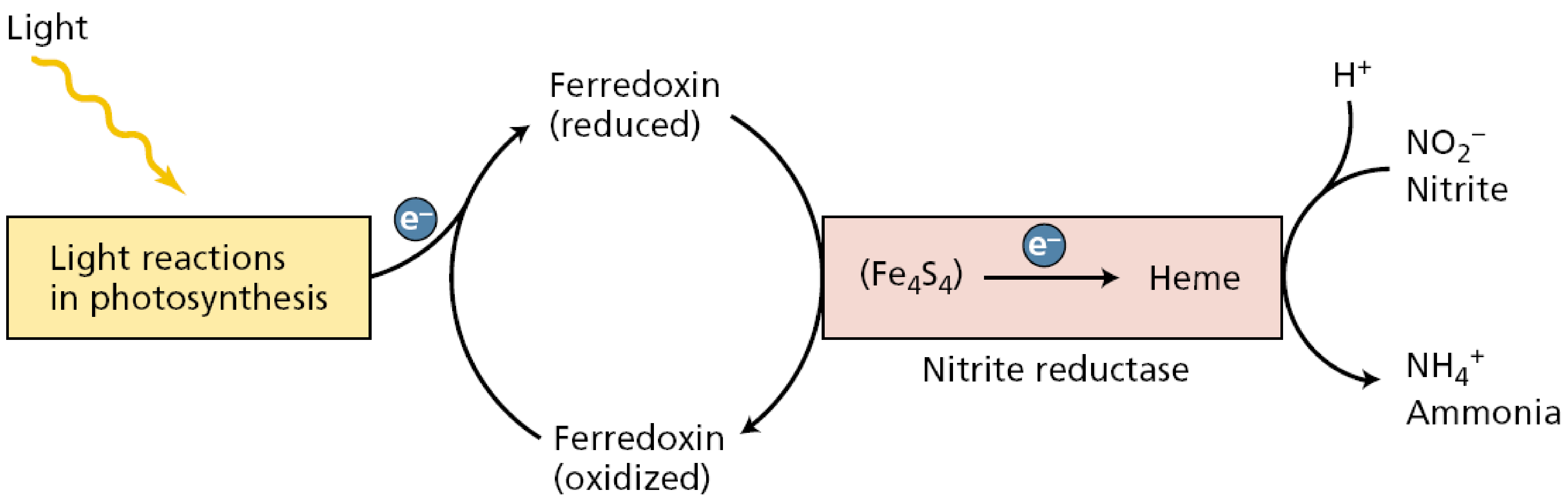


FIGURE 12.5 Model for coupling of photosynthetic electron flow, via ferredoxin, to the reduction of nitrite by nitrite reductase. The enzyme contains two prosthetic groups, Fe_4S_4 and heme, which participate in the reduction of nitrite to ammonium.

Localization in Roots and Shoots

- In most plant species both roots and shoots are capable of nitrate reduction

- The proportion of reduction carried out in each location depends on various factors:
    - Level of nitrate supply
    - Plant species
    - Accompanying cation
    - Leaf age
    - Light

Level of nitrate supply

- When the external nitrate supply is low, a high proportion of nitrate is reduced in the roots
- With an increasing supply of nitarte, the capacity for nitrate reduction in the roots becomes a limiting factor and an increasing proportion of the total nitrogen is translocated to the shoots in the form of nitrate

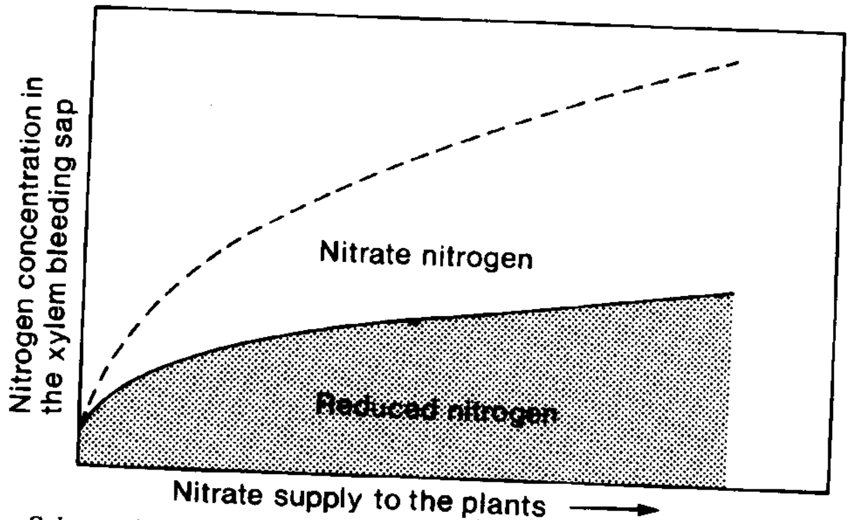


Fig. 8.3 Schematic representation of the effect of the level of nitrate supply in the rooting medium of noninoculated field pea (*Pisum arvensis* L.) on the nitrogen compounds in the xylem sap of decapitated plants. (Data recalculated from Wallace and Pate, 1965.)

Plant species

- The large carbohydrate requirment for nitrate reduction in the roots is certainly one of the factors limiting the capacity of roots for nitrate reduction
- This capacity differs among species and cultivars
- The capacity for nitrate reduction in the roots of woody species is usually very high

Accompanying cation

- The uptake rate of the accompanying cation affects this proportion

- With potassium as the accompanying cation, translocation of both potassium and nitrate to the shoots is rapid, correspondingly, nitrate reduction in the roots is relatively low

- In contrast, when calcium is the accompanying cation, nitrate reduction in the roots is considerably higher

Leaf age

- Maximum activity occurs when the rate of leaf expansion is maximal
- In fully expanded leaves, nitrate reductase activity is usually very low

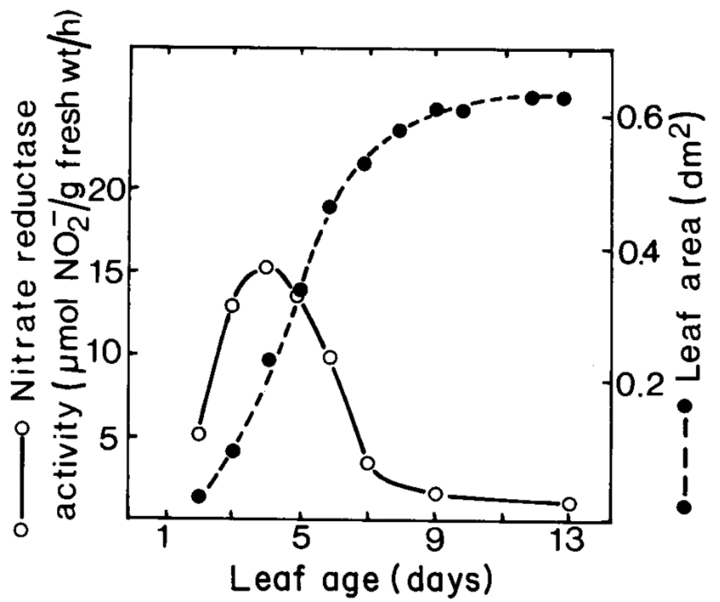
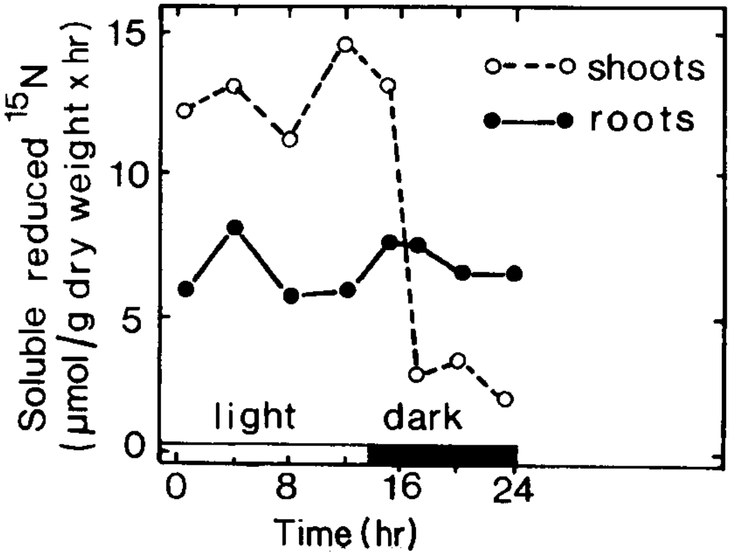


Fig. 8.4 Time course of nitrate reductase activity and leaf area development during the ontogeny of the first trifoliate leaf of soybean. (Modified from Santoro and Magalhaes, 1983.)

Light

- In green leaves a close correlation exists between light intensity and nitrate reduction



. 8.5 Accumulation of soluble reduced ^{15}N in maize during a 24-hr period $^{15}NO_3$ supply to the roots. (Based on Pearson *et al.*, 1981.)

- Light effect in carbohydrate level
- Light effect in feredoxin level

N-Compound with low molecular weight

Low molecular weight organic nitogen compounds which are important storage and long-distance transport forms

Compond	Plant family
Glutamine, Asparagine	Gramineae
Glutamine	Ranunculaceae
Asparagine	Fagaceae
Arginine, Glutamine	Roseaeae
Proline, Allantoin	Papilionacea
Betaine	Chenopodiaceae

Role of low molecular weight nitrogen compounds

- As intermediates between the assimilation of inorganic nitrogen and the synthesis of high-molecular-weight compounds (Such as Proteins)

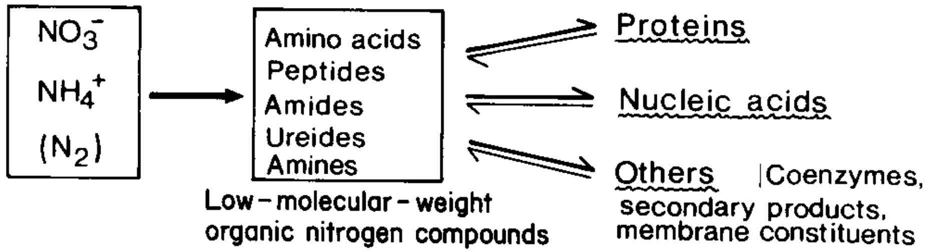


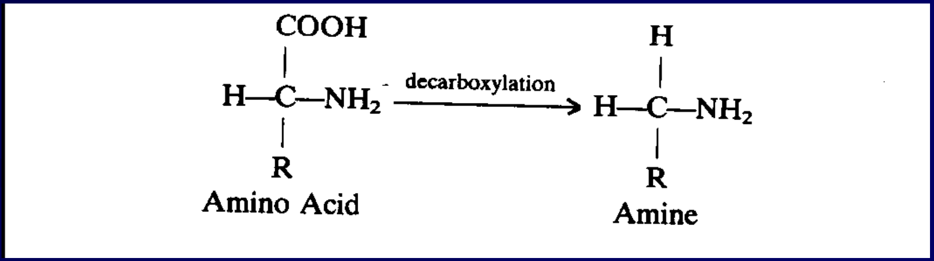
Fig. 8.10 Major classes of nitrogen compounds in plants.

- As intermediates between the assimilation of inorganic nitrogen and the synthesis of hormones (Triptophan to Auxin)

- Involved in the long-distance transport of metal cations in the xylem (such as Cu^+, Mn^+)

- Involved in osmoregulation in plants (Proline, Betaine)

- Involved in redox systems [For example the tripeptide glutathione (Glutamine, Glycine and Cysteine) functions in the redox system of chloroplast and in the long-distance transport of reduced sulfur in the phloem]



- Precursors of amine synthesis

- Involved in nucleic acids synthesis

- Involved in polyamines synthesis

- Synthesis of Heat Shock Proteins

Ammonium Versus Nitrate Nutrition

- Whether plants should be supplied with nitrate- or ammonium-based fertilizers

- Nitrate fertilizer: NO_3^- → Leaching ↓

  Flooding and lack of O_2, NO_3^- --------------→ (Denitrification) N_2↑, N_2O↑

- Ammonium fertilizer: NH_3 ----------------→ (Nitrification, Nitromonase) NO_2^- ------------→ (Nitrobacter) NO_3^-↓

- Nitrification inhibitors: - Synthesis (N-Serve, Dicyano-diamide, Nitropyrin)

  - Naturally compound (Tea)

- For any given plant species, the uptake and utilization of ammonium is greater than that of nitrate.

- In some case, highest growth rates are obtained with a combination of both ammonium and nitrate or with ammonium only.

- Effects of nitrogen source on plant growth are related to the:
  1. Changes in the phytohormonal balance
  2. Cation-Anion balance
  3. Root respiration

Table 6.3
Effect of Ammonium versus Nitrate Supply during the Period of Flower Bud Differentiation on the Flowering of Jonathan Apple Trees in the Following Growing Season[a]

Treatment	No. of buds per tree		Percentage of flower buds
	Emerged buds	Flower buds	
Continuous nitrate N supply	35·5	12·8	38·7
Nitrate N supply interrupted by 8 weeks of ammonium N supply	33·3	21·2	63·7
Nitrate N supply interrupted by 24 hr of ammonium N supply	38·8	30·3	77·5

[a]From Grasmanis and Edwards

- For example: In apple trees flower formation is affected to a much greater by the time and/or form of nitrogen application than by the level of nitrogen supply

- These effects of nitrogen source on flower initiation are related to the changes in the phytohormonal balance.
- This is a supported by results of researches demonstrating that cytokinin export from roots to the shoots in higher with ammonium than with nitrate.

Table 6.4

Effect of Nitrogen Supply on CYT Concentration in Xylem Sap of Apple Root Stocks and on Shoot Growth

Treatment	Zeatin concentration 1 day after start of nitrogen supply (μg/ml)	Shoot growth after 4 months	
		No. of new spurs (<5 cm)	Total length (cm) of the new shoots (>5 cm)
Control (N supply withheld)	0·05	12	16
Ammonium N	1·95	17	34
Nitrate N	0·82	13	48

[a]Based on Buban *et al.* (1978).

- Effect of nitrogen on cytokinin synthesis:

  Directly: Role of nitrogen on cytokinin precursor formation (Isoprene)

  Indirectly: Role of nitrogen on root primordia formation

- Ammonium more effective on cytokinin synthesis than nitrate, due to ammonium more active on both directly and indirectly effects

How nitrogen source affect on flower bud formation?

1. Relation between cytokinin and flower morphogenesis

2. Related to the changes in the polyamines synthesis especially putrescine

Step 1 (wavy arrow): perception of LD induction by mature leaves; step 2 (solid arrow): starch mobilization in leaves and stem followed by transport of sucrose in the phloem to both the apical meristem and roots; step 3 (dashed arrow): transport in the xylem from roots to leaves of zeatin riboside ([9R]Z) and isopentenyladenine riboside ([9R]iP); step 4 (dotted arrow): transport in the phloem from leaves to the apical meristem of isopentenyladenine (iP). RH, relative humidity.

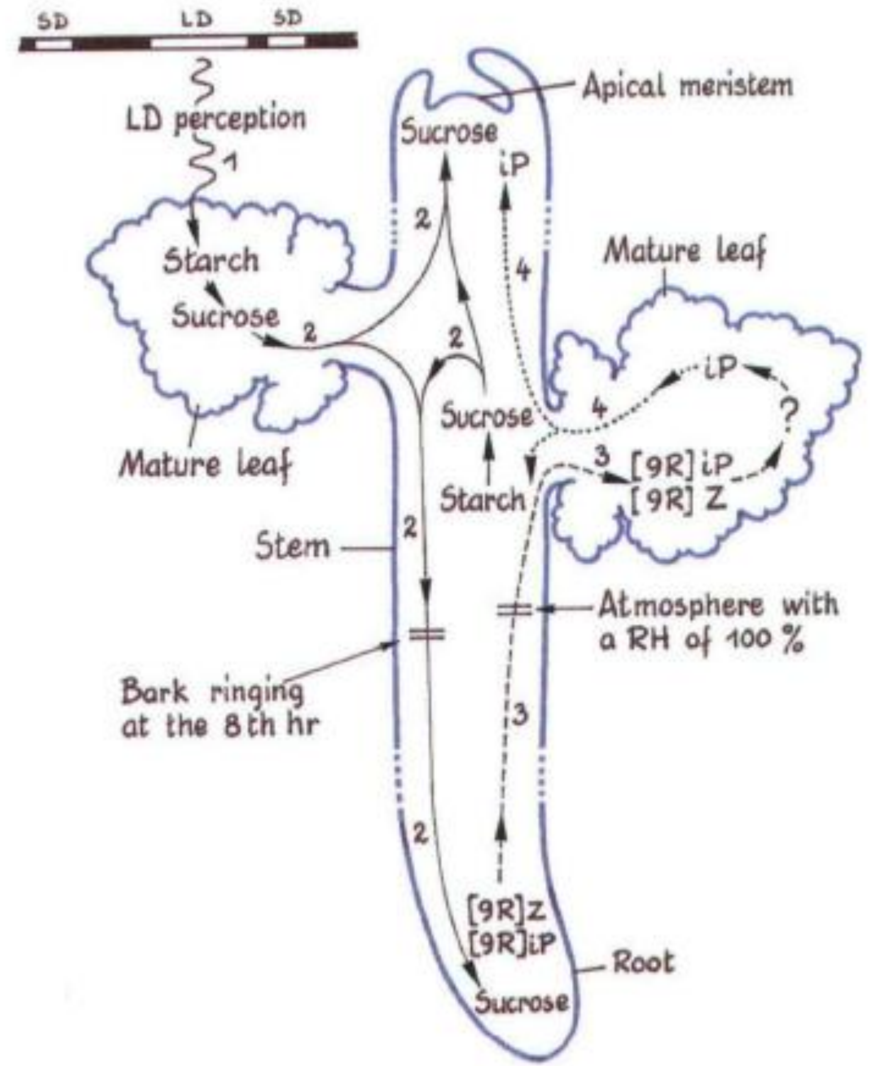


Figure 1. Diagram of a Regulatory Loop Participating in the Control of the Transition to Flowering in *S. alba* and Involving Sucrose and Cytokinins.

Putrescine formation in roots can be increased by ammonium supply

Ammonium (with positive charge) compete with K^{+}, Ca^{+2} and Mg^{+2}

Putrescine synthesis related to disruption of the K^{+}

Table 8.4

Effect of Ammonium and Potassium Supply on Root Growth and Root Putrescine Content in Pea Plants[a]

Treatment: Nitrogen supply (NO_3^-/NH_4^+)	Treatment: Potassium supply	Dry weight of the roots (g)	Putrescine (μmol/g dry wt)
100:0	$+K^+$	134	0·6
	$-K^+$	113	10·3
50:50	$+K^+$	92	24·5
	$-K^+$	80	50·5

[a]From Klein *et al.* (1979).

Because the enzyme systems involved in putrescine synthesis are stimulated by low pH the increase in putrescine level in ammonium-fed plants can be regarded as the response of the roots to low cellular pH and as part of a mechanism for maintaining cellular pH by the synthesis of basic compounds.

Cation-Anion balance

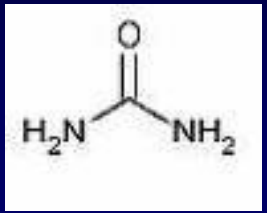
Ammonium generally inhibits cation uptake and can depress growth by inducing a deficiency of magnesium or calcium

Root respiration

- Ammonium, unlike nitrate, increases root respiration
- Ammonium is toxic in high concentrations, therefore it must be trapped into organic molecules
- Ammonium assimilation necessary to energy and carbon skeletons, which both produced in respiration

Urea

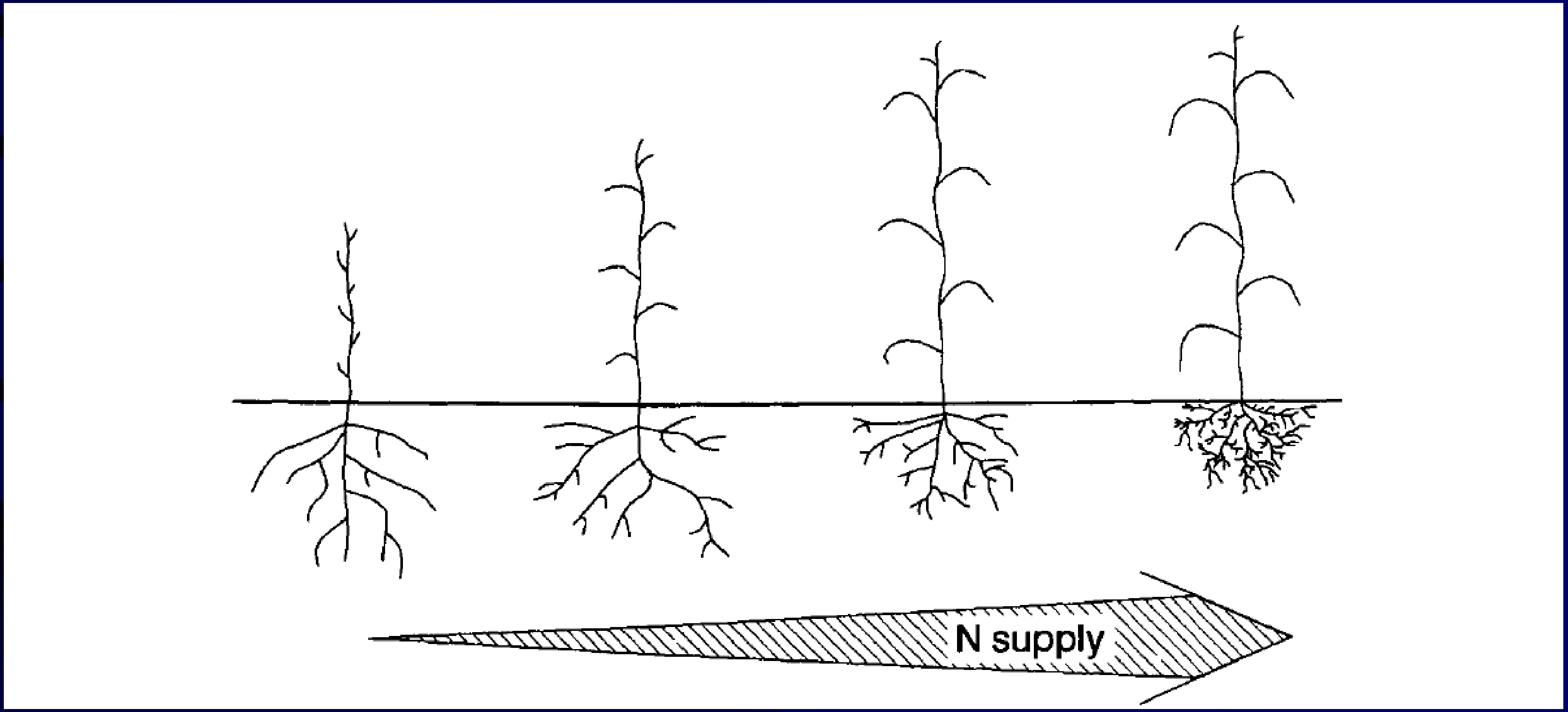
- Urea, another nitrogen source, can be used by plant
- Urea can be taken up directly by the roots or aerial parts
- After being taken up by the roots it is rapidly hydrolyzed by the enzyme urease either within the roots (e. g. in soybean) or after translocation to the shoots (e. g. in maize)
- In soils the hydrolysis of urea usually takes place before root uptake



$$(NH_2)_2CO \xrightarrow{\text{Urease}} CO_2 + 2NH_3$$

Nitrogen supply, plant growth and plant composition

- Depending on the plant species, development stage, and organ, the N content required for optimal growth varies between 2 and 5% of the plant dry weight
- N is mobilized in mature leaves and retranslocated to areas of new growth as amino acid (Aspartic acid, Glutamic acid) and Amines (Aspargin and Glutamin)
- N supply not only delay senescence and stimulates growth but also changes plant morphology
-


N supply

Interacting Effects of Nitrogen Fertilizer Supply and Growth Regulation by Chlorocholine Chloride (CCC) on Lodging

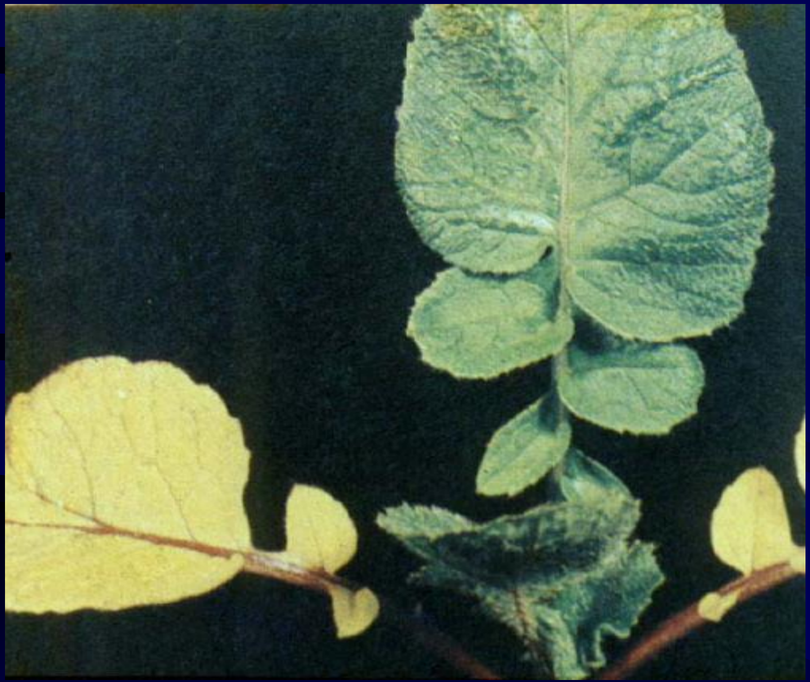
N supply (kg ha^{-1})	Degree of lodging[b]		Grain yield (t ha^{-1})	
	−CCC	+CCC	−CCC	+CCC
0	2.4	1.0	3.97	4.18
80	4.8	1.2	4.71	5.13
120	5.8	1.8	4.67	5.13
160	6.3	1.7	4.80	5.31

Table 8.7
Effect of Increasing Nitrogen Supply as NH_4NO_3 on Dry Matter Production and Composition

	Nitrogen supply (g per pot)			
	0.5	1.0	1.5	2.0
Dry matter (g per pot)	14.9	23.2	26.2	26.0
Composition (% dry wt)				
Total nitrogen	2.0	2.8	3.6	4.2
Sucrose	7.7	7.3	7.1	6.3
Polyfructosans	10.0	4.3	1.8	1.1
Starch	6.1	3.4	2.1	1.4
Cellulose	14.4	13.9	13.9	17.6

Nitosamine

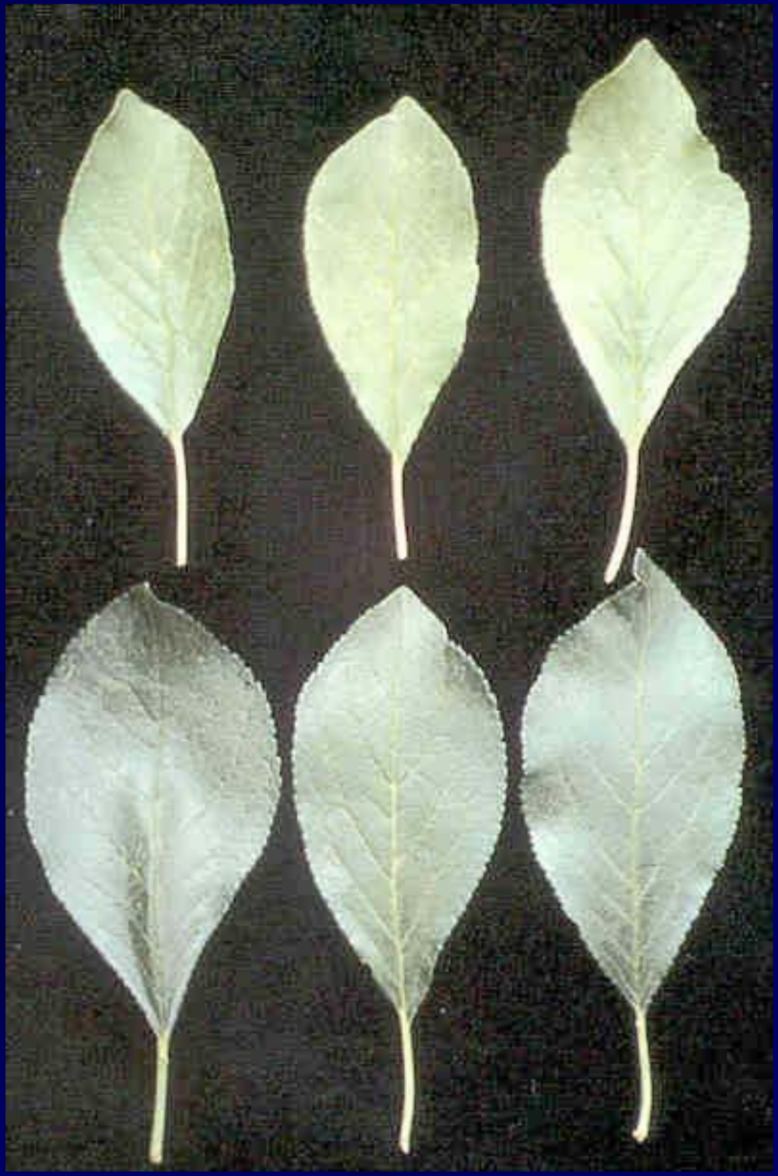
N-deficient rape



Nitrogen Deficiency in Prune

Deficient

Sufficient



N deficieny in grape

Base of stem appears vinicolor in N deficient maize (anthocyanin accumulation)

N Fertilizers

Ammonium Nitrate (24%)

Ammonium sulfate (21%)

Urea (46%)

Calcium Nitrate (15.5%)