N assimilation

Organic N

- Proteins- 20 amino acids
- DNA + RNA- nucleic acids
- Chlorophylls
- Secondary products
- More- hormones

N in the environment

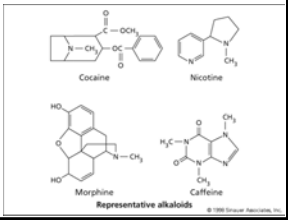
NH_4^+, NH_3 (oxid st -3)

NO_3^- (oxid st +5)

N_2 (oxid st 0)

Next to photosynthesis, the most important role of plants (and microorganisms) in the food chain is the ability to obtain N from the environment.

$R\text{-}CH\text{-}COO^-$ with NH_3^+ on the CH



Human nutrition requirement

Carbohydrate
Fat (linoleic acid)
Minerals

8-10 essential amino acids from proteins: Leu, Ile, Lys, Met, Phe, Thr, Trp, Val, (infants) Arg, His

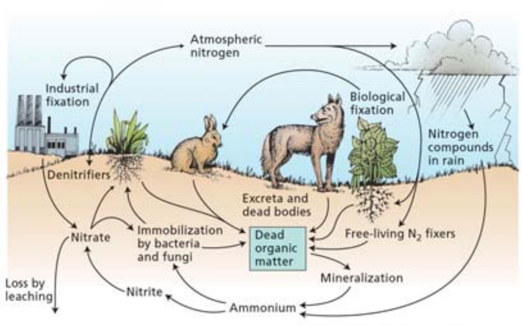
Vitamins: A, B1, B2, B3, B6, B12, C, D, E, K, pantothenic acid, biotin, folic acid

Protein Scores

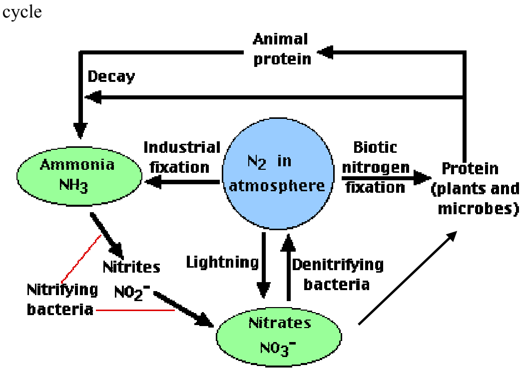
Hen's egg	100
beef	80
cow's milk	79
chicken	72
fish	70
rice	69
soybean	67
wheat	62
maize	49
beans	44
potatoes	34

Low in Trp, Met, Lys

12-1. Taiz. N cycle. Only plants and microbes are able to obtain N from both soil and air



N cycle



http://www.ultranet.com/~jkimball/BiologyPages/N/NitrogenCycle.html

N Assimilation:

Only plants and microorganisms are able to obtain N from both air and soil..

N CYCLE

Two ways plants convert available N to a biological useful form.

I. Biological N Fixation: N_2 ---.> NH_3

II. NO_3^- Reduction: NO_3^- ---> NH_4^+

NH_4^+ --> Amides or Ureides --> Amino acids --> Proteins

NITRATE REDUCTION

All plants can take up N in the form of NH_4^+ and Nitrate (NO_3^-). Nitrate must be reduced to NH_3^+ before it can be incorporated into amino acids, proteins and nucleic acids.

a. Nitrate Reductase

NO_3^- + NADPH + H^+ ---------> NO_2^- + NADP + H_2O

b. Nitrite Reductase

NO_2^- + 6 Fd_{red} + $6H^+$----> NH_4^+ + $2H_2O$

NH_4^+ is assimilated into organic N before it can be exported. NH_4^+ is incorporated into

I. Amides (ASP-NH_2) or

II. Ureides

And then transported elsewhere.

Amides or Ureides --> AMINO ACIDS ---> PROTEINS

Amides or Ureides ---> NUCLEIC ACIDS --> DNA, RNA

NITRATE REDUCTION

All plants can take up N in the form of NH_4^+ and nitrate (NO_3^-). Nitrate must be reduced to NH_3^+ before it can be incorporated into amino acids, proteins and nucleic acids.

1. Nitrate Uptake into plant: H^+/NO_3 symport
   NO_3 is stored in vacuoles for use later
2. Two enzymes reduce NO_3 --> NH_3

a. **Nitrate Reductase**

NO_3^- + NADPH + H^+ ---------> NO_2^- + NADP + H_2O

b. **Nitrite Reductase**

Leaf plastid: NO_2^- + 6 Fd_{red} + $8H^+$----> NH_4^+ + $2H_2O$

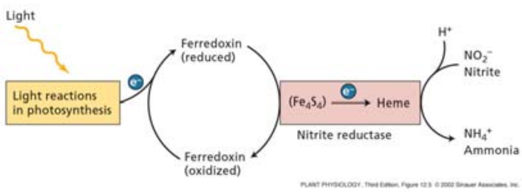
Root cytoplasm: NO_2^- + 3NADPH + $5H^+$-> NH_4^+ + $2H_2O$ + NADP+

Two enzymes use a lot of reducing energy

3. NH_3 or NH_4+ is used directly ---> amino acids

Q? Energy costly. Where does reducing power come from?

12-5. Taiz. Light supplies reducing power to reduce Nitrite.



How is nitrate reductase synthesis and activity regulated?

Synthesis:

NR gene---> mRNA --> NR enzyme

Activity:

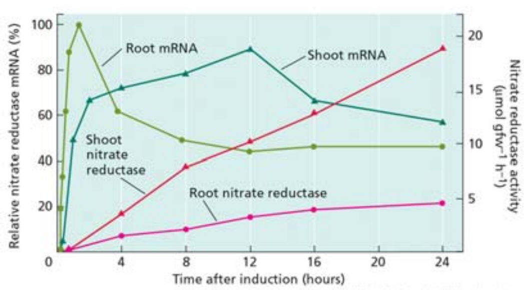
NR enzyme inactive ---> active

Nitrate

Light

Carbohydrate level

12-4. Taiz. Nitrate reductase is induced by NO_3^-



NO_3^-

1. Gene expression and synthesis;

Regulation of NR by nitrate, light & CHO

1. Gene expression is induced by NO_3
2. Modification of enzyme: inactive → active by light

   enzy-ser-OP --Pase --> enzy-ser-OH

   inactive ----------> active
3. In dark-grown seedlings, sucrose or glucose induced NR expression.

Q? How do plants sense N in the soil?
How do plants sense sucrose level?
Why do grasses under the shade show N deficiency?

Mini-review Reference

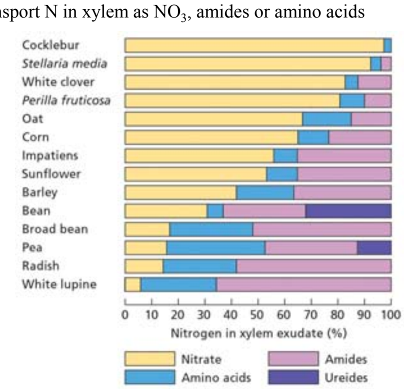
Gloria Coruzzi and Daniel R. Bush

Plant Physiol, January 2001, Vol. 125, pp. 61-64

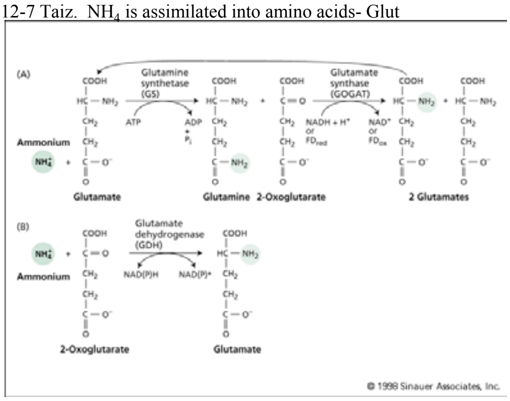
Nitrogen and Carbon Nutrient and Metabolite Signaling in Plants

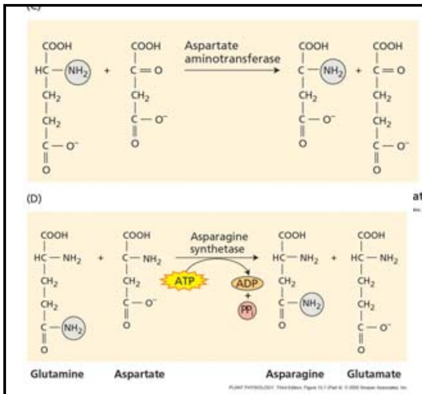
Approach:
Identify all NO3-induced genes using microarray

12-6 Taiz.How do plants transport N?
Plants transport N in xylem as NO_3, amides or amino acids



12-7 Taiz. NH_4 is assimilated into amino acids- Glut





12-7 Taiz

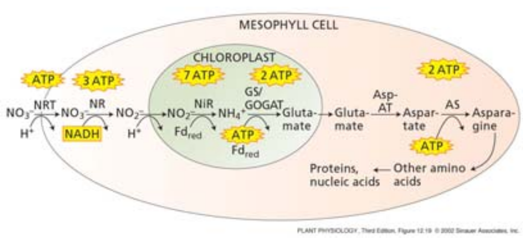
NH_4 in glut is assimilated into asparagine.

Asparagine has 2 NH_2 groups.

Asparagine is an amide.

$2NH_4$ + OAA + NADH +3ATP --> $AspNH_2$ +2ADP +2Pi + NAD+

12-19. Nitrate reduction and NH_4 assimilation need ATP and reducing power. Energy costly

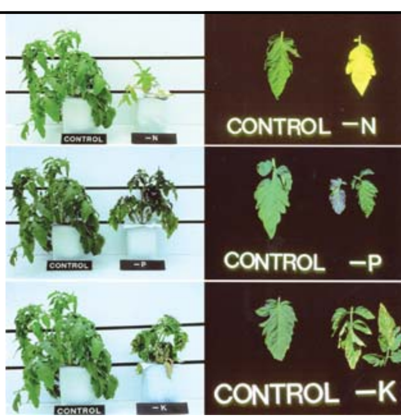


Amides: Glutamine: 5 C and 2 N

Asparagine: 4 C and 2 N

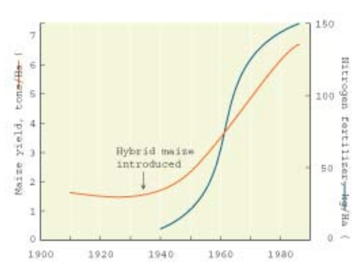
Ureides: some plants make ureides

e.g. allaintoic acid: 4 C and 4 N



ch 3-5 . **N fertilizer Increase Maize Yield in US**

Chrispeels & Sadava 2002



N Assimilation- Summary:

Only plants and microorganisms are able to obtain N from both air and soil..

N CYCLE

Two ways plants convert available N to a biological useful form.

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NH_4^+ is assimilated into organic N before it can be exported. NH_4^+ is incorporated into

I. Amides (ASP-NH_2) or

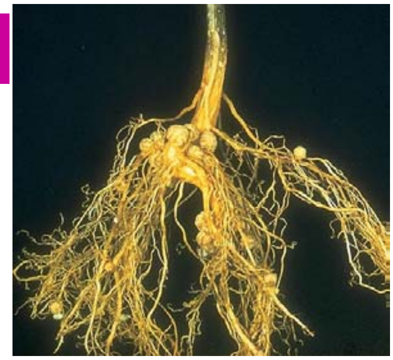
II. Ureides

These are transported elsewhere.

Amides or Ureides --> AMINO ACIDS ---> PROTEINS

Amides or Ureides ---> amino acids -->NUCLEIC ACIDS --> DNA, RNA

Biological N fixation



N_2 fixing
Soybean nodules

Nitrogen Fixation I — Organisms: anatomy, physiology, ecology
http://web.reed.edu/academic/departments/biology/nitrogen/index.html

N_2 fixation -

Two types of biological N fixation

1. Free-living bacteria
2. Symbiotic N fixers. E.g. legume-Rhizobium

Symbiotic N fixation in legume starts when N is limiting in the soil

a. Recognition and binding of bacteria to root
b. Nodulation
c. Bacteroids develop ability to fix N_2

Development is dependent on regulated expression of bacterial and plant genes

Atmospheric N is fixed by one enzyme: Nitrogenase

N_2 + 8e- + $8H^+$ + 16ATP --> $2NH_3$ + H_2 + 16 ADP + 16 Pi

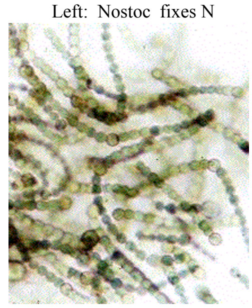
Reducing power: Fd or NADH

NH_4^+ is assimilated into organic N (amides or ureides) and then exported via the xylem.

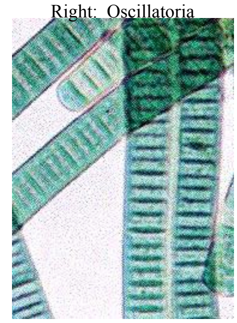
Free-living N-fixing bacteria:

Cyanobacteria: are photosynthetic bacteria.

Left: Nostoc fixes N



Right: Oscillatoria



Symbiotic N fixers: Legume-Rhizobium

Examples of legumes

Phaseolus vulgaris. Green beans

From: TAMU

Soybean

Glycine max flowers

from TAMU

Trifolium repens: white clover (weed)

Medicago sativa

Alfalfa

From: http://www.csdl.tamu.edu/FLORA/cgi/gallery_query?q=medicago+sativa

Table 12-3 Taiz. **Symbiotic N Fixers**.
Specific association between host plant and Rhizobium

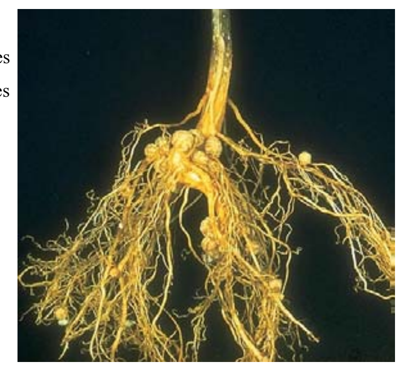
Host Plant	**Rhizobium**
soybean	Bradyrhizobium japonica
Alfalfa (Medicago sativa)	R. meliloti
Pea (Pisum sativum)	R. leguminosarum bv viciae
Clover (Trifolium)	R. leguminosarum bv trifolii

Development depends on regulated expression of plant and bacterial genes

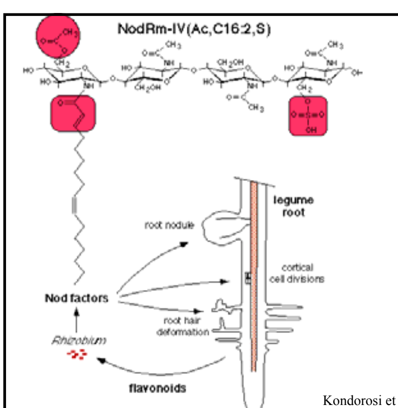
Plant Genes expressed	***Bacteria Genes***
Nodulins	Nif, Nod, Fix

Soybean nodules

How are nodules formed?



Nitrogen Fixation I — Organisms: anatomy, physiology, ecology
http://web.reed.edu/academic/departments/biology/nitrogen/index.html

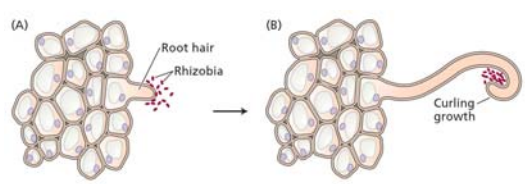


Attraction via chemistry!

Kondorosi et al. http://www.isv.cnrs-gif.fr/ak/index.html

12-11 Taiz. Infection process that leads to N fixation.

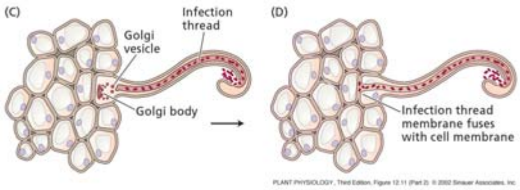
How does it start?



Attraction starts with chemistry!

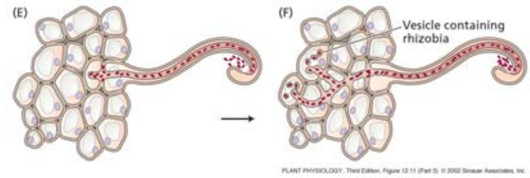
Root hair curls; bacteria divide

12-11



Formation of infection thread from Golgi secretory vesicles

infection thread reaches end of cell

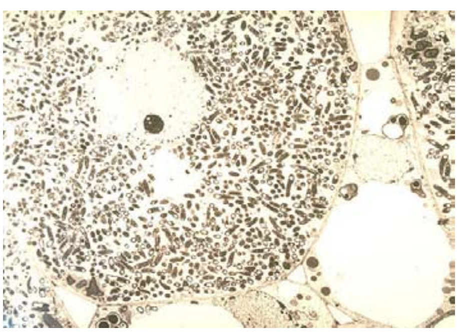


Bacteria released into wall initiate new infection thread

Infection thread reaches target cells and vesicles containing bacteria are released.

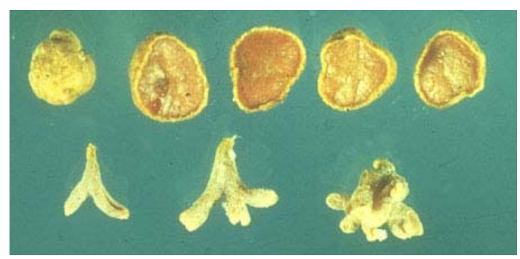
Bacteroids in cortex of root nodule.

Symbiosome:



Nodule section of soybean, alfalfa

NB Leghemoglobin



Atmospheric N is fixed by one enzyme: Nitrogenase in bacteroid

Overall reaction:

$N_2 + 8e^- + 8H^+ + 16ATP \rightarrow 2NH_3 + H_2 + 16\ ADP + 16\ Pi$

Reducing power: Fd or NADH

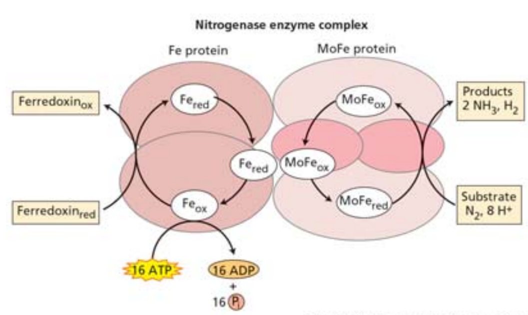
Sum of 2 reactions:

$N_2 + 6e^- + 6H^+ + 12ATP \rightarrow 2NH_3 + 12ADP + 12\ Pi$

$2e^- + 2H^+ + 4ATP \rightarrow H_2 + 4ADP + 4\ Pi$

Where do reducing energy and ATP come from?

12-12 Reaction of Nitrogenase



Where do reducing power & ATP come from?

1. **Electrons**
   Fd
   NADH

2. **ATP**

Nitrogenase activity is inactivated by oxygen.

LegHemoglobin keeps [O_2] low.

Oxygen inactivates the enzyme irreversibly.

Isolated Leghemoglobin

Plants synthesize new proteins in response to nodulation.
Such proteins are nodulins.

Plant gene products of N fixation that are only turned on after nodulation.

LegHb is a plant nodulin.

Enzymes to synthesize amino acids.
 Asparagine synthase

NH_4^+ assimilation:

NH_4^+ is converted to amides or ureides

$2NH_4^+$ + OAA + NADH +3ATP --> $AspNH_2$ +2ADP +2Pi + NAD+

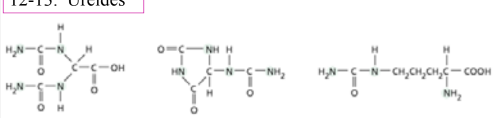
2 Glut + $2NH_4^+$ + 2ATP ------> $2Glut\text{-}NH_2$ + 2ADP + 2Pi

Glut-NH_2 + a-ketoglut -> Glut + Glut
Glut + OAA --> Asp + a-ketoglut

Asp + **Glut**-NH_2 $_{+ATP}$--> $AspNH_2$ + Glut
amide

NH_4^+ assimilation:
NH_4^+ is converted to amides or ureides

12-13. Ureides



4C and 4N

Ureides are then exported via xylem to other organs, cells.

Summary: Getting N into proteins, nucleic acids, chlorophylls & secondary metabolites

NO_3^- / N_2 → NH_4^+ → Amides / Ureides → 20 Amino acids → **Proteins**; **Nuc acids**- RNA & DNA; **Other N cpds**: chlorophyll, NADPH, 2nd products

1. Reduce N to NH_4^+
2. NH_4^+ is used to synthesize amide or ureide. Amides and ureides are transported via xylem to other parts
3. Amides & ureides are converted to other amino acids
4. Amino acids are used for synthesis of proteins, nucleic acids, other N-cpds