N Assimilation
Organic N
- Proteins: 20 amino acids
- DNA + RNA: nucleic acids
- Chlorophylls
- Secondary products
- More: hormones

N in the environment
NH₄⁺, NH₃ (oxid st -3)
NO₃⁻ (oxid st +5)
N₂ (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.

Human nutrition requirement

Carbohydrate
Fat (linoleic acid)
Minerals
8-10 essential amino acids
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

N cycle

NITRATE REDUCTION
All plants can take up N in the form of NH₄⁺ and nitrate (NO₃⁻). Nitrate must be reduced to NH₃⁺ before it can be incorporated into amino acids, proteins and nucleic acids.

1. Nitrate Uptake into plant: H⁺/NO₃ symport
   NO₃ is stored in vacuoles for use later
2. Two enzymes reduce NO₃ --> NH₃⁺
   a. Nitrate Reductase
      NO₃⁻ + NADPH + H⁺ → NO₂⁻ + NADP + H₂O
   b. Nitrite Reductase
      NO₂⁻ + 6Fdₜₚₜ + 8H⁺ → NH₃⁺ + 2H₂O
   NH₃⁺ is assimilated into organic N before it can be exported. NH₃⁺ is incorporated into
   i. Aminos (ASP-NH₂)
   ii. Ureides
   And then transported elsewhere.
   Aminos or Ureides: AMINO ACIDS: PROTEINS
   Aminos or Ureides: NUCLEIC ACIDS: DNA, RNA

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?

<table>
<thead>
<tr>
<th><strong>Synthesis:</strong></th>
<th>NR gene $\rightarrow$ mRNA $\rightarrow$ NR enzyme</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th><strong>Activity:</strong></th>
<th>NR enzyme inactive $\rightarrow$ active</th>
</tr>
</thead>
</table>

Nitrate
Light
Carbohydrate level

12-4. Taiz. Nitrate reductase is induced by NO$_3^-$

Regulation of NR by nitrate, light & CHO

1. Gene expression is induced by NO$_3$.
2. Modification of enzyme: inactive $\rightarrow$ active by light
   enzyme-ser-OP $\rightarrow$ enzy-ser-OH
   inactive $\rightarrow$ 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

Nitrogen and Carbon Nutrient and Metabolite Signaling in Plants

Approach:
Identify all NO$_3$-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. NH₄ is assimilated into amino acids- Glut

\[
2NH₄ + OAA + NADH + 3ATP \rightarrow AspNH₂ + 2ADP + 2Pi + NAD⁺
\]

12-19. Nitrate reduction and NH₄ assimilation need ATP and reducing power. Energy costly

\textbf{Amides}: Glutamine: 5 C and 2 N
Asparagine: 4 C and 2 N

\textbf{Ureides}: some plants make ureides
e.g. allantoinic acid: 4 C and 4 N

\textbf{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.
I. Biological N Fixation: N₂ → NH₃
II. N₂O Reduction: NO₃⁻ → NH₄⁺

NITRATE REDUCTION
All plants can take up N in the form of NH₄⁺ and Nitrate (NO₃⁻). Nitrate must be reduced to NH₃ before it can be incorporated into amino acids, proteins and nucleic acids.

- Nitrate Reductase
  NO₃⁻ + NADPH + H⁺ ———→ NO₂⁻ + NADP + H₂O
- Nitrite Reductase
  NO₂⁻ + 6Fdred + 6H⁺ ———→ NH₄⁺ + 2H₂O

NH₃ is assimilated into organic N before it can be exported. NH₄⁺ is incorporated into:
I. Amides (ASP-NH₂) or
II. Ureides

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

N₂ 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
- Recognition and binding of bacteria to root
- Nodulation
- Bacteroids develop ability to fix N₂
- Development is dependent on regulated expression of bacterial and plant genes

Atmospheric N is fixed by one enzyme: Nitrogenase
N₂ + 8e⁻ + 8H⁺ + 16ATP ———→ 2NH₃ + H₂ + 16 ADP + 16 Pi
Reducing power: Fd or NADH

NH₃ is assimilated into organic N (amides or ureides) and then exported via the xylem.

Cyanobacteria: are photosynthetic bacteria.

Free-living N-fixing 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

<table>
<thead>
<tr>
<th>Host Plant</th>
<th>Rhizobium</th>
</tr>
</thead>
<tbody>
<tr>
<td>soybean</td>
<td>Bradyrhizobium japonica</td>
</tr>
<tr>
<td>Alfalfa (Medicago sativa)</td>
<td>R. meliloti</td>
</tr>
<tr>
<td>Pea (Pisum sativum)</td>
<td>R. leguminosarum bv viciae</td>
</tr>
<tr>
<td>Clover (Trifolium)</td>
<td>R. leguminosarum bv trifolii</td>
</tr>
</tbody>
</table>

Development depends on regulated expression of plant and bacterial genes

- Plant Genes expressed: Nodulins, Nif, Nod, Fix
- Bacteria Genes: Nif, Nod, Fix

Attraction via chemistry!

12-11 Taiz. Infection process that leads to N fixation.
How does it start?
- Attraction starts with chemistry!
- Root hair curls; bacteria divide

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

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:

\[ \text{N}_2 + 8e^- + 8H^+ + 16\text{ATP} \rightarrow 2\text{NH}_3 + H_2 + 16\text{ADP} + 16\text{Pi} \]

Reducing power: Fd or NADH

Sum of 2 reactions:

\[ \text{N}_2 + 6e^- + 6H^+ + 12\text{ATP} \rightarrow 2\text{NH}_3 + 12\text{ADP} + 12\text{Pi} \]

\[ 2e^- + 2H^+ + 4\text{ATP} \rightarrow H_2 + 4\text{ADP} + 4\text{Pi} \]

Where do reducing energy and ATP come from?
Where do reducing power & ATP come from?

1. Electrons
   - Fd
   - NADH

2. ATP

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

\[ \text{NH}_4^+ \text{ assimilation:} \]

\[ 2\text{NH}_4^+ + \text{OAA} + \text{NADH} + 3\text{ATP} \rightarrow \text{AspNH}_2 + 2\text{ADP} + 2\text{Pi} + \text{NAD}^+ \]

\[ 2 \text{Glut} + 2\text{NH}_4^+ + 2\text{ATP} \rightarrow 2\text{Glut-NH}_2 + 2\text{ADP} + 2\text{Pi} \]

\[ \text{Asp} + \text{Glut-NH}_2 + \text{ATP} \rightarrow \text{AspNH}_2 + \text{Glut} \text{ amide} \]

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

1. Reduce N to \( \text{NH}_4^+ \)
2. \( \text{NH}_4^+ \) 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