Nitrogen Assimilation in Plants

Some essential steps for the synthesis of organic matter available to the living world



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Nitrogen: an essential element

- Nitrogen is the fourth most common element
 - Living beings contain a large amount of nitrogen incorporated in proteins, nucleic acids, and many other biomolecules like,
 - PGRs
 - Chlorophyll
 - Cytochromes
 - Flavanoids
 - Alkaloids
 - Coumarins
 - Lignin
 - etc.

• 78% of atmosphere is N2 Most of this is NOT available to living organisms

- **Paradox: limiting** in environment for growth but plenty available in atmosphere as N₂
 - Biologically unavailable!
 - Need prokaryotes to help with this...

Deficiency Symptoms - N

- General chlorosis.
- Chlorosis progresses from light green to yellow.
- Entire plant becomes yellow under prolonged stress.
- Growth is immediately restricted and drop older leaves.



□ During autotrophic growth the nitrogen demand for the formation of cellular matter is met by inorganic nitrogen in two alternative ways:

- 1. Fixation of molecular nitrogen from air; or
- 2. Assimilation of the nitrate or ammonia contained in water or soil.

 \Box Bioavailable forms are nitrate (**NO**₃⁻) and ammonia (**NH**₄⁺)



□ Plants absorb nitrogen from the soil in the form of nitrate (NO3–) and ammonia (NH3).

In aerobic soils where nitrification can occur, nitrate is usually the predominant form of available nitrogen that is absorbed.

Nitrogen Assimilation



Nitrate assimilation is the transformation of simple inorganic nitrogen substances into complex organic nitrogenous compounds like:

- Amino acids,
- Chlorophylls
- Cytochromes
- Flavanoids
- Alkaloids
- Coumarins
- Lignin
- □ This organic nitrogen is present in oxidation state –III (-3; as in NH3).
- Other organisms, like animals, depend entirely on organic nitrogen from their food.





Figure 16.34 Nitrate assimilation by plant cells involves transport of nitrate across the plasma membrane and then reduction to ammonia in a two-step process. A proton-pumping ATPase maintains the electrochemical gradient that drives cellular uptake of nitrate. The values shown for electrical potentials and intracellular nitrate concentrations are typical but can vary



- Requires large input of energy
- Forms toxic intermediates
- Mediated by specialized enzymes that are closely regulated.
- The nitrate taken up into the root cells can be stored there temporarily in the vacuole.
- Nitrate is reduced to NH_4^+ in the epidermal and cortical cells of the root.
- This NH_4^+ is mainly used for the synthesis of glutamine and asparagine.

Nitrate Assimilation: The reduction of nitrate to NH_4^+ ; proceeds in two partial reactions:

Nitrate Assimilation

(Green plants, some fungi and bacteria)



- Nitrate is assimilated in the leaves and also in the roots.
- Many woody plants (e.g., trees, shrubs), as well as legumes such as soybean, assimilate nitrate mainly in the roots.

Nitrate Assimilation:

$$NO_3^- + NADH + H^+$$

Nitrate Reductase $NO_2^- + H_2O + NAD^+$

□ The nitrate reductases of higher plants are composed of two identical sub-units, each containing three prosthetic groups

- FAD—flavin adenine dinucleotide
- Heme
- Molybdenum—organic molecule called pterin









Nitrite Reductase Converts Nitrite to Ammonium

• Nitrite (NO₂⁻)is highly reactive

 $NO_2^- + 8H^+ + 6e^-$ *Nitrite Reductase* $NH_4^+ + 2H_2O$

- Plant cells immediately transport the nitrite generated by nitrite reduction from the cytosol into chloroplasts in leaves and plastids in roots
 - In these organelles, nitrite reductase reduces nitrite to ammonium



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Ammonia Assimilation:

- □ Conversion of ammonia generated from nitrate assimilation or
- □ photorespiration into amino acid.
- □ 2 pathways i. Primary Pathway
 - ii. Alternative Pathway

Primary Pathway:

- 2 enzymes involved :
- a) Glutamine synthetase (GS) Available in two forms- in cytosol, and root plastid/
- shoot chloroplast.
- b) Glutamate synthase (also known as GOGAT)
- GOGAT: Glutamine 2-oxo-glutarate aminotransferase

Glutamine synthetase action

Cytosolic form -

- Expressed in germinating seeds or in vascular bundle of roots and shoots.
- Produce glutamine for intracellular nitrogen transport.

Root plastid/Shoot chloroplast form –

- In roots, it produces amide nitrogen for local consumption.
- In shoots, it reassimilates photorespiratory NH₄⁺



Types of GOGAT

NADH-GOGAT

- accepts electrons from NADH.
- Iocated in plastids of non-photosynthetic tissue like roots or VB of developing leaves.

Glutamine + 2-oxoglutarate + NADH + H⁺

2 Glutamate + NAD+

Fd-GOGAT

- accepts electron from ferrodoxin.
- Iocated in chloroplast and serves in photorespiratory nitrogen metabolism.

Alternative Pathway:

□ The enzyme involved is Glutamate Dehydrogenase (GDH), hence known as GDH pathway.

□ Catalyzes synthesis and deamination of glutamate.

□ Reversible



Transamination Reaction:

Transfer of amino group of an amino acid to α-keto acid resulting in formation of new amino acid and new keto acid.

Catalyzed by Transaminase (Aminotransferase)

- Co-factor: Pyridoxal phosphate(Vitamin B6)
- □ Reversible



Role of Pyridoxal Phosphate(PLP)



Pyridoxal Phosphate:

- □ Serves as a carrier of amino group.
- □ Transfer of alpha- amino group to PLP forms Pyridxamine Phosphate, and a keto acid.
- □ Alpha- amino group is finally transferred to acceptor keto acid to form a new amino acid.





Origin of Carbon Skeletons for the synthesis of Amino Acids