Chapter II : Metabolism of Macronutrients (N, P, K)

1. Nitrogen (N) : Nitrogen cycle, biological N_2 fixation, nitrification and denitrification. Assimilation mechanisms (GS/GOGAT pathway)

In soil, nitrogen occurs mainly in two forms: inorganic nitrogen, which represents about 2%, and organic nitrogen, which accounts for approximately 98%. Inorganic nitrogen includes ammonia (NH_3), ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-). Organic nitrogen is present in living organic matter, such as soil microorganisms and fresh plant and animal residues, as well as in non-living organic matter, including humified and non-humified compounds. Mineral nitrogen is directly available to plants mainly in the form of ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N). In contrast, organic nitrogen is not directly available to plants and must first be converted into ammonium and/or nitrate through the slow process of mineralization.

1.1. Nitrogen cycle

The global biochemical nitrogen cycle involves a series of redox reactions that serve either for nitrogen assimilation or for energy conservation through respiratory pathways. Assimilatory processes include nitrogen fixation, in which atmospheric nitrogen (N_2) is converted into ammonia by free-living or symbiotic bacteria, and assimilatory nitrate reduction, in which nitrate is reduced to ammonium. Both processes produce ammonium, which is incorporated into carbon skeletons and used for the growth of organisms.

In addition to the respiratory reduction of nitrate to ammonia, which occurs widely among anaerobic fermentative bacteria, two major energy-generating processes are involved in the nitrogen cycle : nitrification and denitrification. During nitrification, soil bacteria and other nitrifying microorganisms oxidize ammonia to nitrate. During denitrification, anaerobic or facultative anaerobic microorganisms reduce nitrate to N_2 and other nitrogen gases under oxygen-limited or anaerobic conditions.

Two other microbial processes contribute to the completion of the global nitrogen cycle : ammonification and anammox. Ammonification involves the decomposition of organic matter by bacteria and fungi, releasing nitrogen in mineral forms that can be reused by other organisms. Anammox, or anaerobic ammonium oxidation, is a process in which ammonium is oxidized using nitrite, resulting in the production of nitrogen gas (N_2).

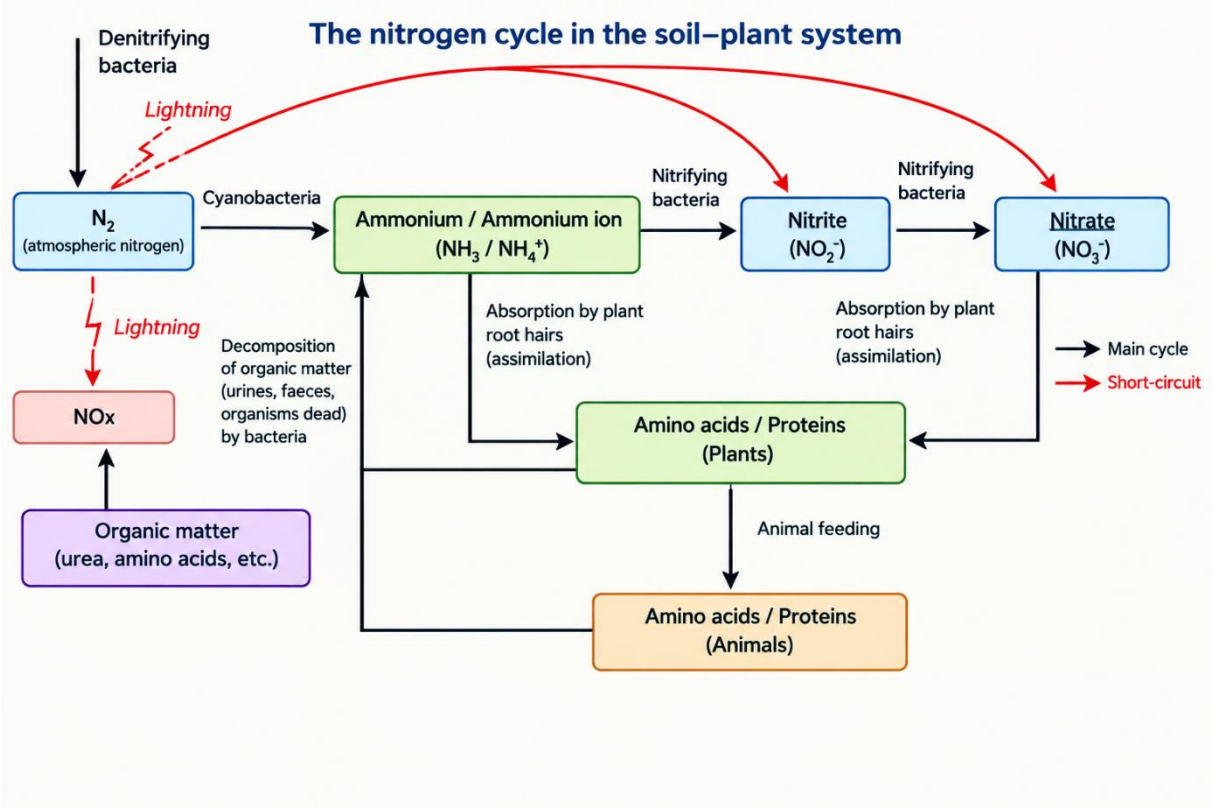

Figure 1 : Simplified Structure of the Nitrogen Cycle.

1.2. Biological N_2 fixation

Biological nitrogen fixation (BNF) is the process by which atmospheric dinitrogen (N_2) is converted into a plant-usable form, primarily ammonium (NH_4^+). During this process, N_2 is reduced using hydrogen derived from water. Nitrogen fixation is not exclusively a biological process, as natural phenomena such as lightning and fire can also contribute to nitrogen transformation by oxidizing atmospheric N_2 and producing nitrogen oxides that can ultimately form nitrate (NO_3^-).

Biological nitrogen fixation is essential for the nitrogen supply of ecosystems, as organisms depend directly or indirectly on this process to obtain nitrogen. Fixed nitrogen is required for the synthesis of nucleic acids, proteins, and numerous other nitrogen-containing organic compounds.

Nitrogen fixation is an energetically demanding process. The reduction of one N_2 molecule requires approximately 16 ATP molecules, while additional energy is needed for the assimilation and transport of NH_4^+. Overall, the process may require approximately 28 ATP molecules. In symbiotic associations, nitrogen-fixing plants provide carbohydrates, such as glucose, to their bacterial partners in exchange for biologically fixed nitrogen.

Nitrogen fixation is catalyzed by the enzyme complex nitrogenase, which is highly conserved among nitrogen-fixing bacteria. Nitrogenase consists of two main components: the molybdenum–iron protein (MoFe protein), which contains the catalytic site, and the iron protein (Fe protein), which supplies electrons required for nitrogen reduction. These components are encoded mainly by the *nifD*, *nifK*, and *nifH* genes.

Three types of nitrogenase have been identified according to the metal present in their active center: Mo-nitrogenase, V-nitrogenase, and Fe-nitrogenase, containing FeMo-, FeV-, and FeFe-cofactors, respectively. Nitrogenase is highly sensitive to oxygen, which can inactivate the enzyme and inhibit nitrogen fixation. V-nitrogenase and Fe-nitrogenase are generally more sensitive to oxygen than Mo-nitrogenase.

Because nitrogenase is highly sensitive to oxygen, nitrogen-fixing microorganisms have developed different mechanisms to protect the enzyme from oxygen inhibition. Many diazotrophic bacteria perform nitrogen fixation under anaerobic or microaerophilic conditions. Cyanobacteria have developed specialized strategies to separate nitrogen fixation from oxygen production. In heterocyst-forming cyanobacteria, such as *Nostoc* and *Anabaena*, nitrogen fixation occurs in specialized cells called heterocysts. These cells have thick walls that limit oxygen diffusion and protect nitrogenase. In non-heterocystous cyanobacteria, nitrogen fixation is temporally separated from photosynthesis and mainly occurs in darkness, when oxygen production is greatly reduced.

Some nitrogen-fixing bacteria, such as *Azotobacter*, have developed mechanisms to protect nitrogenase from oxygen. They maintain a low intracellular oxygen concentration through a high respiratory rate. Under high oxygen levels, nitrogenase can also adopt a protected, temporarily inactive state, which helps prevent irreversible damage. In *Azotobacter vinelandii*, the production of alginate capsules around the cells provides an additional protective mechanism by acting as a barrier that limits oxygen diffusion into the cell.

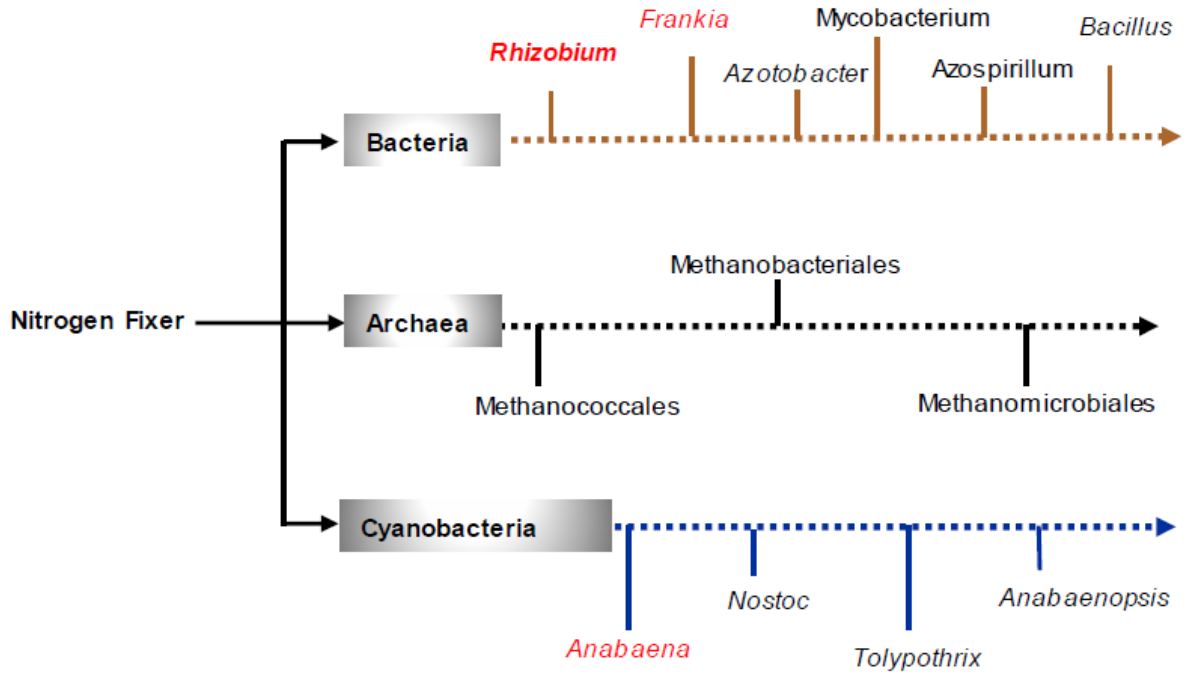

Figure 2 : The three major groups of nitrogen-fixing organisms, including representative genera. Genera containing symbiotic nitrogen-fixing species are shown in red, whereas orders or genera (in italics) containing free-living nitrogen-fixing species are shown in black.

1.3. Nitrification and denitrification

1.3.1. Nitrification

Nitrification is an essential process in the nitrogen cycle of soils, natural waters, and wastewater treatment systems. It involves the biological conversion of ammonium (NH_4^+) into nitrate (NO_3^-), two nitrogen forms that can be used by plants but have different behaviors and environmental fates. Ammonium is mainly produced through the decomposition of organic matter and is often a preferred nitrogen source for plants and algae. Nitrate, in addition to serving as a nutrient, can be used by denitrifying bacteria and converted into dinitrogen gas (N_2), which is generally unavailable to most plants.

The overall nitrification process can be expressed by the following equation :

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$$

Nitrifying Microorganisms

-Ammonia-Oxidizing Bacteria (AOB)

Nitrification is carried out by two functional groups of microorganisms, collectively known as nitrifiers. The first group, ammonia-oxidizing bacteria (AOB), oxidizes ammonia (NH_3) to nitrite (NO_2^-), representing the first step of nitrification. In natural waters, nitrogen is mainly present as ammonium (NH_4^+), whereas NH_3 is the form directly used as the substrate by the enzyme responsible for ammonia oxidation. The oxidation of ammonium by ammonia-oxidizing bacteria, such as *Nitrosomonas*, can be represented by the following overall reaction:

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+$$

AOB are generally obligate chemoautotrophs that obtain energy from ammonia oxidation and use carbon dioxide (CO_2) as their main carbon source. Their major metabolic product is nitrite, while nitrous oxide (N_2O) can be produced as a minor by-product.

- Ammonia-Oxidizing Archaea (AOA)

Ammonia-oxidizing archaea (AOA) are a distinct group of microorganisms involved in the first step of nitrification. They were first successfully cultivated in 2005. Like ammonia-oxidizing bacteria (AOB), AOA oxidize ammonia (NH_3) to nitrite (NO_2^-), but they use different enzymatic pathways. Most AOA are considered autotrophic and fix CO_2 through the 3-hydroxypropionate/4-hydroxybutyrate pathway rather than the Calvin cycle.

AOA are widely distributed in marine and terrestrial environments and often occur in greater abundance than AOB. Despite differences in their enzymes and metabolic pathways, both groups carry out aerobic ammonia oxidation according to the same overall stoichiometry :

$$NH_3 + 1.5\ O_2 \rightarrow NO_2^- + H_2O + H^+$$

Both AOA and AOB can also produce nitrous oxide (N_2O), an important greenhouse gas. In AOB, N_2O can be formed through nitrifier denitrification or from hydroxylamine (NH_2OH), an intermediate of ammonia oxidation. Nitrifier denitrification is particularly favored under low-oxygen conditions. In contrast, the mechanisms responsible for N_2O production in AOA remain unclear and differ from those of AOB, as AOA do not use hydroxylamine as an intermediate and lack the enzymes involved in nitrifier denitrification.

-Anammox Organisms

Anammox organisms are a specialized group of bacteria belonging mainly to the phylum *Planctomycetes*. They perform anaerobic ammonium oxidation (anammox), in which ammonium (NH_4^+) is oxidized using nitrite (NO_2^-) as the electron acceptor, producing dinitrogen gas (N_2) rather than nitrate. The overall reaction is:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$$

Anammox is a strictly anaerobic process and leads to the removal of fixed nitrogen from ecosystems, making it functionally similar to denitrification. Anammox bacteria are slow-growing autotrophs that fix CO_2 and possess a specialized internal compartment called the anammoxosome, where the reaction takes place. They also contain unique membrane lipids called ladderanes, which help retain the reactive intermediate hydrazine within the cell. The nitrite used in anammox can originate from aerobic ammonia oxidation or partial denitrification.

-Nitrite-Oxidizing Bacteria (NOB)

Nitrite-oxidizing bacteria (NOB) form the second functional group of nitrifying microorganisms. They oxidize nitrite (NO_2^-) to nitrate (NO_3^-), completing the nitrification process. The overall reaction is:

$$2NO_2^- + O_2 \rightarrow 2NO_3^-$$

Well-known genera such as *Nitrobacter* are mainly chemolithoautotrophic, using nitrite as an energy source and CO_2 as their carbon source through the Calvin cycle. Another important genus, *Nitrospina*, is particularly abundant in marine environments and fixes CO_2 through the reductive tricarboxylic acid pathway. Some NOB can also use organic compounds as an additional carbon source and are therefore considered mixotrophic or facultative autotrophs.

- Heterotrophic Nitrifiers

Heterotrophic nitrification is carried out by certain bacteria and fungi that can oxidize ammonium through pathways involving either inorganic or organic intermediates. Unlike autotrophic nitrification, this process does not generate energy for ATP production and generally occurs at much lower rates.

Heterotrophic nitrification may become important under environmental conditions that inhibit autotrophic nitrifiers, such as acidic soils or the presence of inhibitory compounds produced by plants. However, its overall quantitative importance in terrestrial and aquatic ecosystems remains uncertain.

Significance of Nitrification

The significance of nitrification can be summarized as follows :

-Conversion of ammonium into nitrate, influencing nitrogen availability for plants and algae.

-Production of nitrate, which serves as a substrate for denitrification.

-Contribution to nitrous oxide (N_2O) production in aquatic and terrestrial ecosystems.

-Consumption of oxygen, particularly in aquatic sediments.

-Acidification of soils and other environments.

Environmental Factors Affecting Nitrification

Nitrification is influenced by several environmental factors, including temperature, salinity, light, organic matter, substrate availability, pH, and oxygen concentration.

-**Ammonium and nitrite availability :** Ammonium (NH_4^+) is the main substrate for AOB and AOA. Its low concentration in many oxic environments can limit nitrification. AOA generally have a higher affinity for ammonium than AOB, allowing them to remain active at very low concentrations.

-**Oxygen concentration :** AOB, AOA, and NOB require O_2 for nitrification and are mainly found in oxic environments. However, they can tolerate and remain active at low oxygen concentrations. In contrast, anammox microorganisms are strictly anaerobic and their activity is inhibited by oxygen.

-**Organic matter :** Most nitrifiers are autotrophic and obtain carbon from CO_2 rather than organic matter. Therefore, they have little direct competition with heterotrophic microorganisms for organic substrates. Their slow growth is related to the relatively low energy yield of nitrification.

-**Light :** Light can inhibit nitrification, particularly in AOB and NOB, and AOA may also be highly light-sensitive. This sensitivity may influence the distribution of nitrifying microorganisms in aquatic environments.

-**pH :** Nitrification is sensitive to pH because ammonia oxidation produces H^+ and can acidify the environment. Low pH can inhibit AOB and AOA activity, making pH particularly important in acidic soils.

-**Temperature and salinity :** Nitrification occurs across a wide range of temperatures and salinities because different nitrifying microorganisms are adapted to different conditions. However, changes in salinity can strongly affect nitrification rates and the composition of nitrifying communities.

1.3.2. Denitrification

Denitrification is the microbial reduction of soil nitrate (NO_3^-) to gaseous nitrogen compounds, mainly nitric oxide (NO), nitrous oxide (N_2O), and nitrogen gas (N_2). It is carried out mainly by heterotrophic bacteria that use nitrate as an alternative terminal electron acceptor when oxygen (O_2) is limited. This process is favored under water-saturated conditions, such as after

rainfall, in wetlands, rice soils, and within oxygen-depleted soil aggregates or decomposing organic matter. The general pathway is :



Nar: nitrate reductase, converts NO_3^- to NO_2^-.

Nir: nitrite reductase, converts NO_2^- to NO.

Nor: nitric oxide reductase, converts NO to N_2O.

Nos: nitrous oxide reductase, converts N_2O to N_2.

- **Denitrifier Diversity**

-Denitrification is performed by a wide range of soil microorganisms, including organotrophs, chemo- and photolithotrophs, N_2-fixing bacteria, thermophiles, halophiles, and some pathogenic species.

-More than 50 genera and 125 denitrifying species have been identified.

-Most culturable soil denitrifiers are facultative anaerobes, mainly belonging to *Pseudomonas* and *Alcaligenes*, with smaller contributions from *Bacillus*, *Agrobacterium*, and *Flavobacterium*.

-Denitrifiers generally represent about 0.1–5% of the culturable soil microbial population and may account for up to 20% of total microbial biomass.

-Denitrifying microorganisms generate energy (ATP) through electron transport phosphorylation, using nitrate and its reduction products as terminal electron acceptors under oxygen-limited conditions.

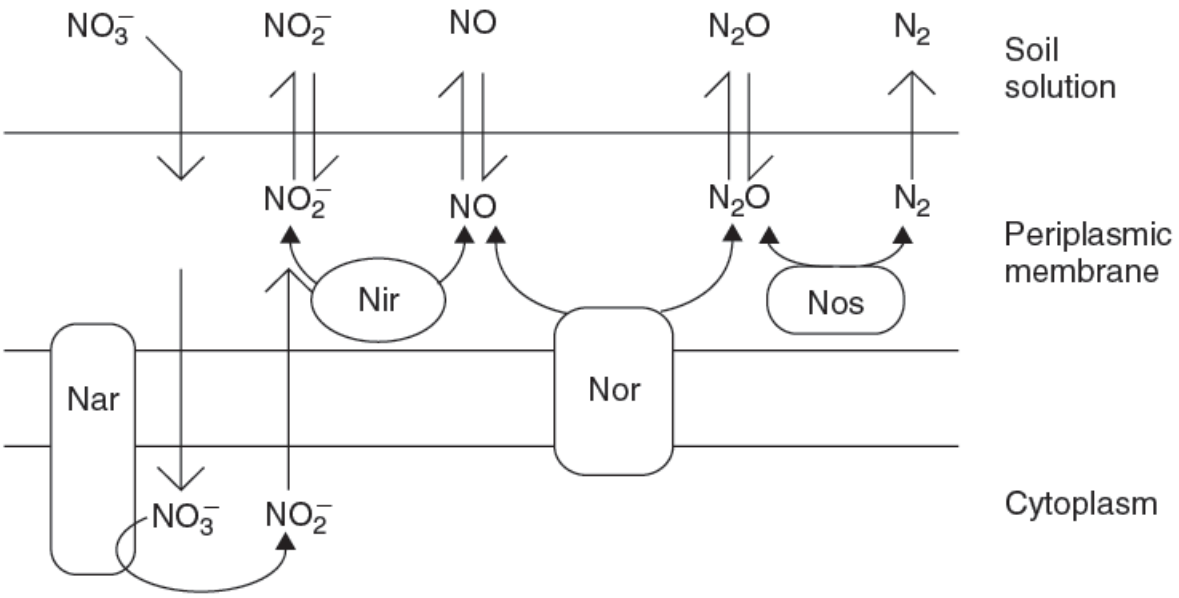

Figure 3 : The organization of denitrification enzymes in the cell membrane for gram negative Bacteria.

- **Significance of Denitrification**

-Completes the nitrogen cycle by returning fixed nitrogen to the atmosphere mainly as N_2.

-Removes excess nitrate (NO_3^-) from soils, groundwater, and wastewater, helping to reduce nitrate pollution.

-Plays an important role in wastewater treatment by converting nitrate into gaseous nitrogen.

-Can cause nitrogen losses from agricultural soils, reducing nitrogen availability for plant uptake.

-Produces N_2O, a potent greenhouse gas that also contributes to stratospheric ozone depletion.

1.4. Assimilation Mechanisms (GS/GOGAT pathway)

Assimilatory nitrate reduction is an important physiological process and represents one of the major pathways through which inorganic nitrogen is incorporated into carbon skeletons in higher plants, algae, including cyanobacteria, and fungi. Nitrate assimilation is a multistep process that begins with nitrate uptake and its subsequent reduction to ammonium by two highly regulated enzymes: cytosolic nitrate reductase (NR) and plastidic nitrite reductase (NiR). The resulting ammonium is incorporated into carbon skeletons using 2-oxoglutarate supplied by the tricarboxylic acid (TCA) cycle, followed by amino acid transamination reactions that produce glutamate and glutamine. These two amino acids serve as the main nitrogen donors for the biosynthesis of other nitrogen-containing compounds and secondary metabolites. The

formation of glutamine and glutamate occurs through the coordinated action of glutamine synthetase and glutamate synthase, known as the GS/GOGAT cycle.

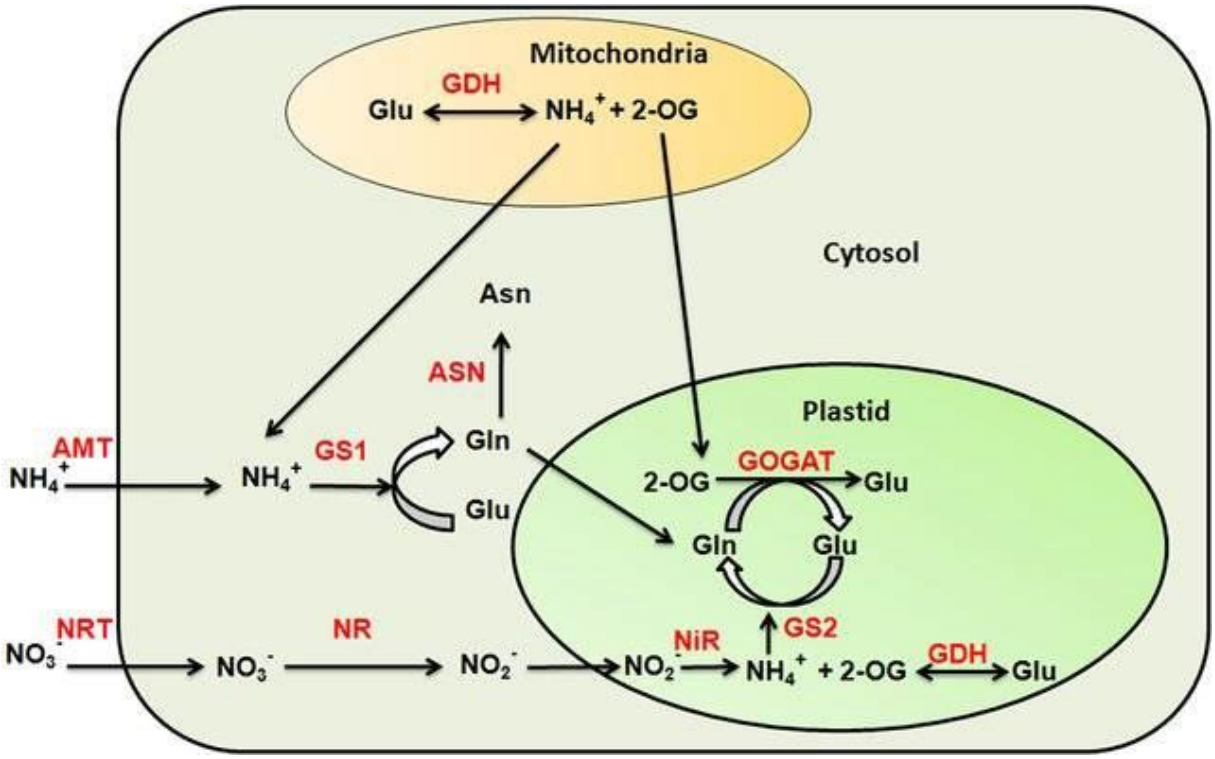

Figure 4 : Nitrate Transport and Assimilation : Nitrate and ammonium are absorbed by root cells through nitrate transporters (NRTs) and ammonium transporters (AMTs), respectively. Nitrate is reduced to nitrite by nitrate reductase (NR), while nitrite is subsequently reduced to ammonium by nitrite reductase (NiR) in the plastids. The resulting ammonium is then incorporated into carbon skeletons through the coordinated action of glutamine synthetase and glutamate synthase.

The interconversion of glutamate and glutamine is essential for nitrogen assimilation, transport, recycling, and maintaining nitrogen homeostasis in plants. The two key enzymes involved are glutamine synthetase (GS) and glutamate synthase (GOGAT). Roots are a major site of ammonium (NH_4^+) assimilation, where GS incorporates NH_4^+ into glutamate to form glutamine. Subsequently, GOGAT transfers the amino group from glutamine to 2-oxoglutarate (2-OG), producing glutamate.

The coordinated activity of GS and GOGAT is therefore essential for efficient ammonium assimilation and plant growth. The expression and activity of specific GS/GOGAT isoenzymes increase in response to NH_4^+ availability, indicating that plants regulate this pathway according to their nitrogen status.

Glutamine synthetase (GS) is a highly conserved enzyme that plays a central role in nitrogen metabolism. It catalyzes the ATP-dependent incorporation of ammonium (NH_4^+) into glutamate to produce glutamine. Glutamate synthase (GOGAT) then transfers the amino group from glutamine to 2-oxoglutarate (2-OG), producing two molecules of glutamate. Together, GS and GOGAT form a major pathway for ammonium assimilation in plants.

Plants possess two main forms of GOGAT, which differ in their electron donors. Ferredoxin-dependent GOGAT (Fd-GOGAT) is mainly found in chloroplasts of photosynthetic tissues, where it uses reduced ferredoxin generated through photosynthetic activity. NADH-dependent GOGAT (NADH-GOGAT) is more abundant in non-photosynthetic tissues and uses NADH as the reducing agent. The two forms show different patterns of expression depending on the plant tissue and its metabolic requirements.

- **Isoforms of GS and GOGAT**

Plant glutamine synthetase (GS) occurs mainly in two isoforms based on its subcellular localization. GS1 is predominantly located in the cytoplasm and is mainly involved in nitrogen recycling, whereas GS2 is located in plastids and is primarily associated with the assimilation or reassimilation of ammonium in leaves. GS1 is encoded by multigene families, while GS2 is generally encoded by a smaller number of nuclear genes.

The relative contribution of GS1 and GS2 varies depending on the plant species, organ, nitrogen source, developmental stage, and environmental conditions. In addition, plants contain several GOGAT isoforms, including ferredoxin-dependent and NADH-dependent forms, with specific isoenzymes being expressed in roots in response to ammonium availability.

Overall, the different GS and GOGAT isoforms allow plants to regulate ammonium assimilation and nitrogen recycling according to tissue type and physiological or environmental conditions.

2. Phosphorus (P) : Forms of Phosphorus in Soil and the Role of Mycorrhizal Symbioses in the Uptake of Organic and Inorganic Phosphorus

In their natural state, soils contain approximately one to three tonnes of total phosphorus (P) in the upper 20 cm of soil. This amount greatly exceeds the annual phosphorus requirements of plants. However, only a small fraction, known as bioavailable phosphorus, can be directly used by plants. Bioavailable phosphorus is mainly present in the soil solution as orthophosphate ions, which are readily taken up by plant roots.

Most of the phosphorus potentially available to crops is adsorbed onto soil particles and is gradually released into the soil solution, where it can be absorbed by roots. Adsorbed phosphates are generally associated with cations and with iron (Fe) and aluminum (Al) oxides or hydroxides. Their availability varies considerably: some are rapidly released into the soil solution, whereas others are transferred more slowly from the solid phase to the soil solution or can only be solubilized through the activity of living organisms, such as microorganisms and fungi.

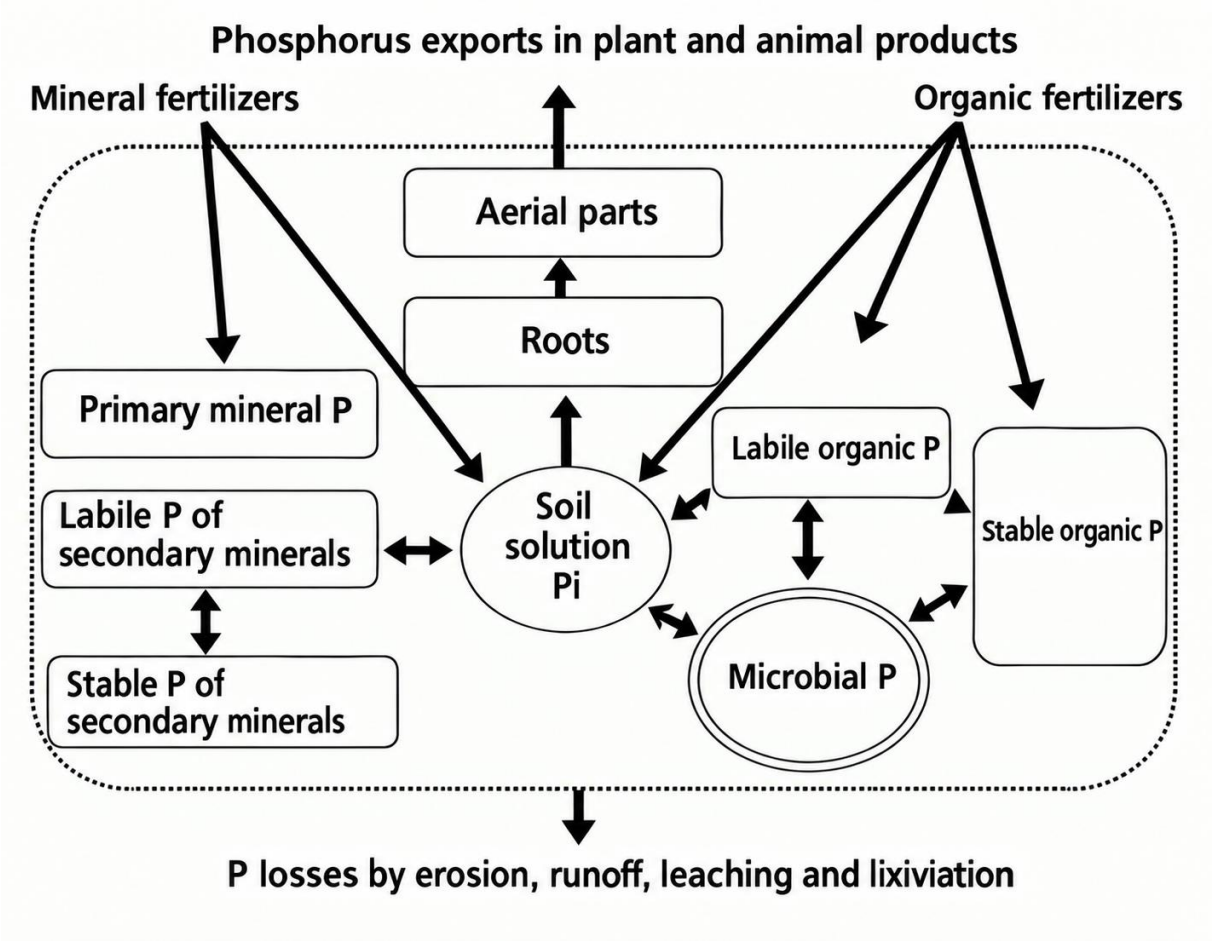

Figure 5 : Phosphorus cycle in the soil/fertilizer/plant system.

2.1. Forms of Phosphorus in Soils

Phosphorus in soils occurs mainly in two forms: inorganic phosphorus (Pi) and organic phosphorus (Po). These forms differ in their chemical properties, behavior, transformation, and availability to plants.

2.1.1. Organic Phosphorus in Soils

Organic phosphorus (Po) represents an important pool of soil phosphorus, although its concentration varies widely among soil types. It mainly originates from decomposing plant and

animal residues, as well as soil microorganisms and fauna. Phosphorus added through fertilizers can also be converted from inorganic to organic forms by microorganisms, particularly when phosphorus availability is low.

Soil organic phosphorus occurs mainly as stable forms, such as inositol phosphates and phosphonates, and more active forms, such as orthophosphate monoesters and organic polyphosphates. It becomes available to plants through mineralization, mainly mediated by soil microorganisms. Mineralization involves the release of inorganic phosphate (Pi) from organic compounds through phosphatase enzymes and the decomposition of organic materials during microbial activity. These processes are strongly influenced by soil moisture, temperature, and the physical and chemical properties of the soil.

2.1.2. Inorganic Phosphorus in Soils

Inorganic phosphorus (Pi) occurs mainly as phosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) adsorbed onto iron (Fe) and aluminum (Al) oxides and hydroxides, associated with organic matter, or bound to calcium (Ca) and magnesium (Mg). Its adsorption and availability are strongly influenced by soil pH and clay minerals.

At low pH, phosphorus tends to form poorly soluble compounds with Fe and Al, whereas near-neutral pH, it forms more soluble compounds with Ca and Mg. At higher pH, phosphorus can again form insoluble calcium phosphates. Therefore, phosphorus availability to plants is generally greatest around pH 6.0–6.5 in most agricultural soils.

- **Calcium and Magnesium Phosphates**

Calcium (Ca) and magnesium (Mg) phosphates are generally absent or unstable in strongly acidic soils. They become more stable and less soluble under neutral to alkaline conditions. The main forms found in soils include :

-Monocalcium phosphate : $Ca(H_2PO_4)_2 \cdot H_2O$, water-soluble and commonly present in superphosphate fertilizers; it can gradually transform into less soluble phosphates.

-Dicalcium phosphate : $CaHPO_4 \cdot 2H_2O$ and $CaHPO_4$, slightly soluble forms, occurring in hydrated and anhydrous forms.

-Octacalcium phosphate : $Ca_8H_2(PO_4)_6 \cdot 5H_2O$, an intermediate calcium phosphate.

-Tricalcium phosphate : $Ca_3(PO_4)_2$ — poorly soluble.

-Hydroxyapatite : $Ca_{10}(PO_4)_6(OH)_2$ and fluorapatite: $Ca_{10}(PO_4)_6F$, stable, highly insoluble calcium phosphates.

-Struvite : $MgNH_4PO_4 \cdot 6H_2O$, a magnesium ammonium phosphate that can occur under alkaline conditions and is relatively soluble.

- **Iron and Aluminium Phosphates**

Iron (Fe) and aluminium (Al) phosphates are important forms of inorganic phosphorus, particularly in acidic soils. Their solubility and stability are strongly influenced by soil pH.

-Aluminium phosphates mainly include variscite ($AlPO_4 \cdot 2H_2O$) and wavellite [$Al_3(PO_4)(OH)_3 \cdot 5H_2O$]. Variscite is common in slightly acidic soils, while aluminium hydroxyphosphates can form at higher pH and regulate phosphorus concentration in the soil solution.

-Iron phosphates are mainly represented by strengite ($FePO_4 \cdot 2H_2O$), which is particularly stable and poorly soluble under acidic conditions. It can coexist with hydrated iron oxides over a range of acidic pH values.

-Under strongly acidic conditions, phosphorus can precipitate as minerals of the variscite and strengite groups, reducing its availability in the soil solution.

-The crystallinity of these minerals affects phosphorus availability: less-crystalline aluminium phosphates generally have a larger surface area and can release phosphorus more readily.

- **Soil Solution Phosphorus**

Soil solution phosphorus refers to the phosphate ions dissolved in the soil solution. It is continuously in equilibrium with the labile phosphorus pool and represents the fraction directly available for plant uptake. Phosphorus concentrations in the soil solution are generally very low, ranging from about 0.001 to 1 mg P L^{-1}, with an average of approximately 0.05 mg P L^{-1}. Plants and mycorrhizal fungi mainly absorb phosphorus as $H_2PO_4^-$ and HPO_4^{2-}, with the dominant form depending on soil pH :

-Below pH 7.2 : $H_2PO_4^-$ is the predominant form.

-Between pH 7.2 and 12.1 : HPO_4^{2-} becomes predominant.

-At very high pH (10.0–14.0) : PO_4^{3-} becomes increasingly dominant.

2.2. Role of Mycorrhizal Symbioses in the Uptake of Organic and Inorganic Phosphorus

Mycorrhizal fungi form mutualistic associations with plant roots. The fungi obtain carbohydrates from the plant, while they improve the uptake of water and mineral nutrients, particularly phosphorus (P). Arbuscular mycorrhizal fungi (AMF) form symbioses with most terrestrial plants and play an important role in P acquisition.

AMF enhance phosphorus uptake through two complementary pathways :

-Root pathway : P is absorbed directly by root hairs and epidermal cells.

-Hyphal pathway : fungal hyphae extend beyond the root surface and penetrate small soil pores that roots and root hairs cannot reach, increasing the volume of soil explored for P.

AMF can also improve P availability by releasing organic acids and phosphatase enzymes, which help solubilize poorly available phosphate compounds. Their hyphae therefore increase the soil volume explored, mobilize otherwise unavailable P, and facilitate its transfer to plant roots.

Under low-P conditions, plants may also increase root hair development, lateral root formation, root length, and root density near the soil surface. Overall, mycorrhizal symbiosis is an important strategy that enables plants to improve phosphorus acquisition, particularly in P-deficient soils.

Phosphorus (Pi) uptake by plants through arbuscular mycorrhizal fungi (AMF) involves several coordinated transport steps :

1-Soil → fungal hyphae : AM fungi absorb Pi from the soil using high-affinity phosphate transporters such as PHO84.

2-Inside the fungus : Pi is rapidly converted into polyphosphate (polyP), which acts as both a phosphorus reserve and a transport form.

3-Fungal transport : PolyP is transported through the fungal hyphae toward the root and then converted back into Pi.

4-Fungus → plant : At the arbuscule, fungal transporters release Pi into the periarbuscular space.

5-Plant uptake : Plant PHT1 phosphate transporters then absorb Pi into the cortical cell cytoplasm.

3. Potassium (K) : Role in Osmoregulation, Stomatal Opening, and Enzyme Activation

Potassium (K^+) is an essential macronutrient that plays a central role in plant cell physiology. It constitutes approximately 2.6% of the Earth's crust, making it the seventh most abundant element and the fourth most abundant mineral nutrient in the lithosphere. In soils, potassium primarily originates from the weathering and decomposition of potassium-bearing rocks and minerals. Major primary minerals that serve as sources of K include orthoclase, microcline, muscovite, and biotite. Potassium is also present in secondary clay minerals, particularly illite, vermiculite, and chlorite, which contribute to the soil K pool. Overall, potassium is an important

constituent of the Earth's crust and is generally more abundant in igneous rocks than in sedimentary rocks.

Potassium occurs mainly in four forms :

-**Water-soluble K :** Potassium present in the soil solution as a soluble cation (K^+) is referred to as water-soluble K. It is readily available for plant uptake because it is not strongly held by cation exchange sites. However, this form of K is highly mobile and may be subject to leaching, depending on soil properties. Water-soluble K accounts for approximately 10% of the available potassium in soil.

-**Exchangeable K :** Exchangeable potassium is the form of K held on the surfaces of soil colloids and can be replaced by other cations present in the soil solution. It is readily available for plant uptake and constitutes approximately 90% of the available potassium in soil. However, exchangeable K generally represents less than 2% of the total potassium content of the soil.

-**Non-exchangeable K :** Non-exchangeable potassium is not readily available for plant uptake. However, it remains in equilibrium with the available forms of potassium and therefore serves as an important reservoir of slowly available K. Certain clay minerals can fix potassium within their layered structures. During soil wetting and drying, K^+ ions may become trapped between the mineral layers. When the soil is rewetted, some of the trapped K^+ is released into the soil solution and becomes available to plants. This slowly available form of potassium is generally not determined by routine soil testing.

-**Lattice K :** Lattice potassium is the fraction of potassium structurally incorporated within the crystal lattice of 2:1 clay minerals. It represents approximately 92–98% of the total potassium in many soils. The high proportion of lattice K is mainly associated with the presence of K-bearing minerals in the soil. This form of potassium is released slowly through mineral weathering, and the rate of release depends on soil texture and environmental conditions.

Plants use several types of membrane proteins to regulate K^+ uptake, transport, and loss. The main groups involved in K^+ nutrition are K^+-selective channels, KT/HAK/KUP transporters, and HKT proteins.

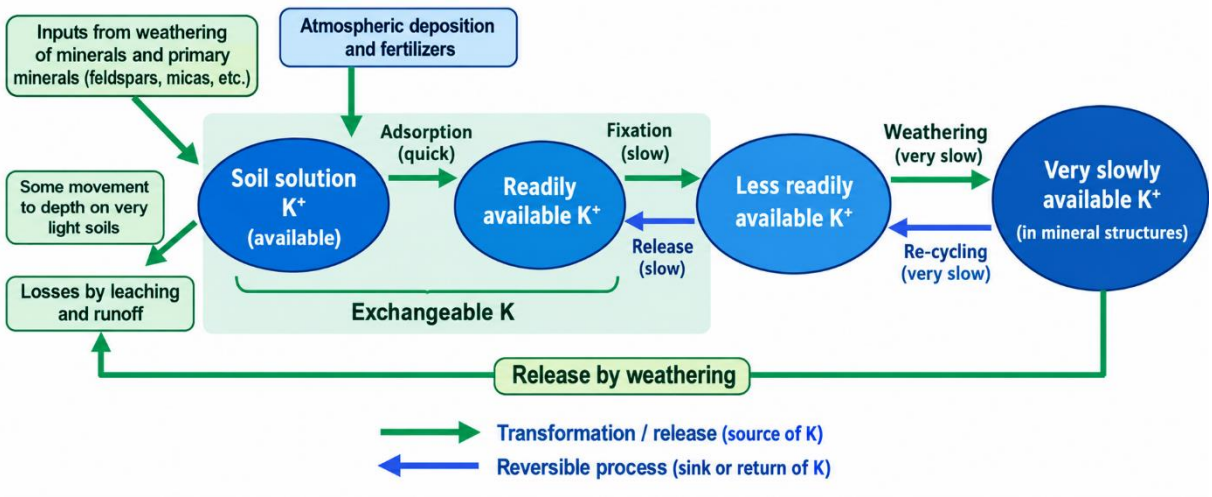

Figure 6 : Simplified Potash Cycle.

K^+-Selective Channels

K^+ channels provide a pathway for K^+ to move across the membrane according to its electrochemical gradient. The first plant K^+ channel identified as being involved in nutrient uptake was AKT1, a Shaker-like voltage-gated K^+ channel.

Plant voltage-gated K^+ channels are generally composed of four α-subunits that form a central pore. Each subunit contains six transmembrane segments (S1–S6). Segments S1–S4 form the voltage-sensor domain, whereas S5, S6, and the pore loop form the K^+-permeation pore.

According to their response to membrane potential, plant K^+ channels can be divided into three groups :

-Inward-rectifying channels (Kin): open mainly at hyperpolarized membrane potentials and promote K^+ uptake. Examples include AKT1, AKT6, KAT1, and KAT2.

-Outward-rectifying channels (Kout): open mainly at depolarized potentials and promote K^+ efflux. The main examples are SKOR and GORK.

-Weakly rectifying channels (Kweak): can mediate both K^+ uptake and release. AKT2 is an example.

KC1 (KAT3) is an electrically silent channel that can associate with AKT1 and other inward-rectifying channels. In roots, the AKT1/KC1 complex contributes to efficient K^+ uptake while limiting K^+ leakage, particularly under low-K^+ conditions.

AKT1 activity is regulated by signaling proteins, particularly the CBL–CIPK23 complex, which activates the channel under K^+ deficiency. Other regulatory proteins, including PP2C phosphatases and CBL10, can also modulate AKT1 activity.

GORK, an outward-rectifying channel, has an important role in K^+ efflux. In guard cells, GORK-mediated K^+ loss contributes to stomatal closure. In roots, particularly under salt and oxidative stress, excessive GORK activity can promote K^+ loss from cells.

KT/HAK/KUP K^+ Uptake Transporters

The KT/HAK/KUP family represents an important group of K^+ transporters found in plants, fungi, and bacteria. In plants, these proteins are mainly involved in K^+ uptake and cellular K^+ homeostasis.

They have diverse functions, including:

-high-affinity K^+ uptake from the soil;

-K^+ transport between plant tissues;

-regulation of water and osmotic balance;

-responses to drought and salinity;

-root hair development and other developmental processes.

The HAK1-like transporters, belonging mainly to clade I, are particularly important for high-affinity K^+ uptake under low external K^+ concentrations. Examples include AtHAK5, HvHAK1, OsHAK1, OsHAK5, and LeHAK5.

KT/HAK/KUP proteins are membrane transporters containing approximately 10–14 transmembrane segments. Unlike K^+ channels, they function as transport proteins and are especially important when external K^+ concentrations are very low.

HKT Proteins

HKT (High-affinity K^+ Transporter) proteins constitute another group of plant ion transport proteins. Despite their name, many HKT proteins primarily transport Na^+ rather than K^+.

Two major HKT subfamilies have been identified :

-HKT1: mainly Na^+-selective and involved in Na^+ transport and recirculation through vascular tissues.

-HKT2: found mainly in monocots and can transport K^+. Depending on the protein and conditions, HKT2 proteins may function as Na^+/K^+ symporters or K^+-selective transporters.

Therefore, some HKT2 proteins, particularly in cereals, can contribute to K^+ nutrition.

K^+ Uptake by Plant Roots

Root K^+ uptake involves two major transport systems depending on external K^+ concentration.

External K^+ concentration	Main transport system	Main proteins
Low K^+ (<1 mM)	High-affinity uptake	HAK1-like transporters + AKT1-like channels
High K^+ (>1 mM)	Low-affinity uptake	Mainly K^+ channels

At relatively high K^+ concentrations, K^+ can enter root cells through channels because the electrochemical gradient favors its movement into the cell. At very low K^+ concentrations, high-affinity transporters are required to efficiently acquire K^+ from the soil.

High-affinity K^+ uptake can be divided into :

-NH_4^+-sensitive uptake, mainly associated with HAK1-like transporters.

-NH_4^+-insensitive uptake, mainly associated with AKT1-like K^+ channels.

Thus, AKT1-like channels and HAK1-like transporters are the major systems responsible for K^+ acquisition under K^+-deficient conditions.

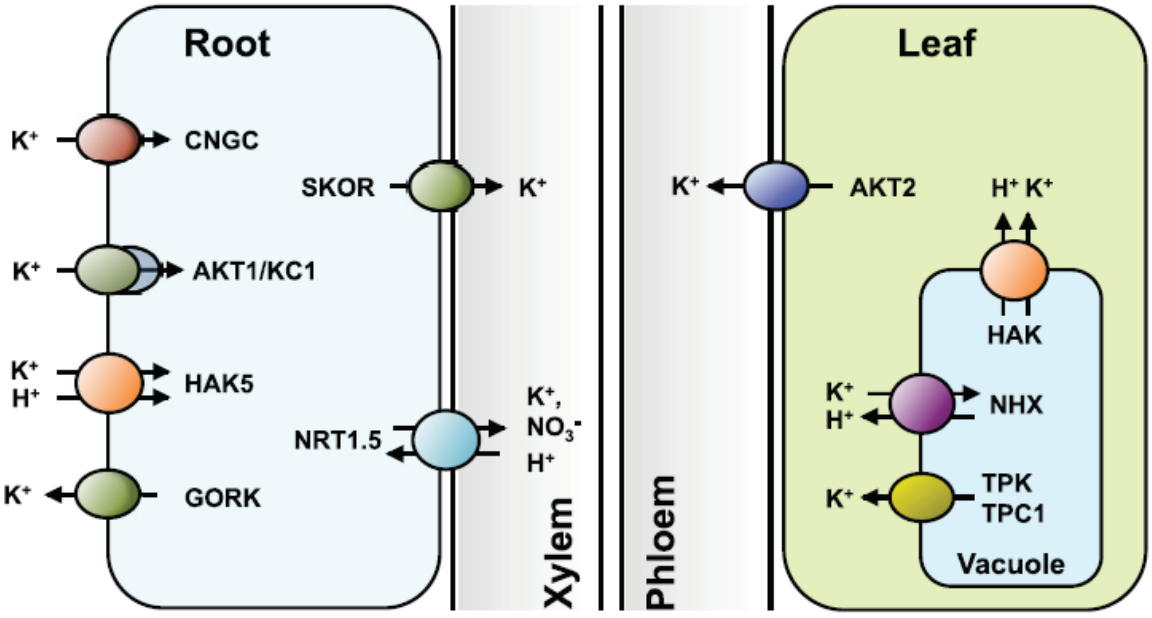

Figure 7 : Transporters involved in K^+ uptake and distribution in plants. K^+ is taken up by roots through HAK5, AKT1, and CNGC channels, transported to shoots through the xylem via SKOR and NRT1.5, and absorbed by aerial tissues through K^+ channels and KT/HAK/KUP transporters. K^+ is stored in vacuoles through NHX exchangers and released by TPK and TPC1 channels. AKT2 contributes to K^+ recycling through the phloem and its redistribution between plant organs.

3.1. Osmoregulation

Potassium (K^+) plays a central role in maintaining plant-cell homeostasis, particularly through the regulation of osmotic balance, water relations, and ion distribution. In plant cells, K^+ concentrations are generally maintained at about 50 mM in the cytoplasm, whereas vacuolar K^+ concentrations can vary considerably. Plants can redistribute K^+ between cytoplasmic and vacuolar pools to maintain a relatively stable cytosolic K^+ concentration.

K^+ is a major osmoticum and contributes directly to osmotic adjustment. In epidermal cells, inorganic ions, particularly K^+, play an important role in maintaining cellular water balance and turgor. In mesophyll cells, although organic osmolytes contribute substantially to osmotic adjustment, K^+ remains an important osmoticum. During the early stages of water deficit, K^+ is a major contributor to osmotic adjustment, while organic osmolytes become increasingly important as water stress progresses.

K^+ homeostasis also involves the redistribution of K^+ between different cellular compartments and tissues. K^+ can be transferred between epidermal and mesophyll cells and between the cytoplasm and vacuole, allowing plants to maintain appropriate cytosolic K^+ levels under changing environmental conditions.

Therefore, K^+ is essential for maintaining cellular osmotic homeostasis, regulating water balance and turgor, and controlling the transport of water, nutrients, and metabolites throughout the plant.

3.2. Stomatal Regulation

Potassium (K^+) plays a key role in the regulation of stomatal opening and closing, thereby controlling gas exchange, photosynthesis, plant cooling, and water loss. In guard cells, K^+ uptake increases the osmotic concentration, promoting water uptake and increasing turgor pressure, which causes the stomata to open. Under water-deficit conditions, K^+ is released from guard cells, leading to a decrease in turgor and stomatal closure, thereby reducing transpiration and protecting the plant from water stress.

Stomatal opening involves three main processes : activation of the plasma-membrane H^+-ATPase, uptake of solutes such as K^+ and accompanying anions (Cl^- and malate), and synthesis or accumulation of organic solutes such as sucrose. These processes lower the osmotic potential of guard cells and promote water uptake. In contrast, stomatal closure is mainly regulated by abscisic acid (ABA), which promotes Ca^{2+} signaling, inhibits K^+ uptake, and stimulates K^+ efflux. The resulting loss of solutes and water decreases guard-cell turgor, causing the stomata to close.

3.3. Enzyme Activation

- **Photosynthesis**

Potassium (K^+) acts as an important activator of several enzymes involved in photosynthesis and plant metabolism. Adequate K^+ availability supports the activity of enzymes associated with photosynthetic processes and carbohydrate metabolism. In particular, K^+ is involved in the activation of ATP synthase, which contributes to ATP production during photosynthesis. Optimal K^+ levels are therefore important for maintaining efficient ATP synthase activity and supporting photosynthetic energy metabolism.

K^+ also influences the activity of RuBisCO, the key enzyme responsible for CO_2 fixation. K^+ deficiency can reduce RuBisCO activity, leading to a decline in photosynthetic CO_2 assimilation. In addition, insufficient K^+ reduces the utilization and export of photoassimilates, which can negatively affect carbohydrate metabolism and plant growth.

- **Nutrient Balance**

Potassium (K^+) is essential for the activity of enzymes involved in nitrogen and carbohydrate metabolism. Under K deficiency, protein synthesis is impaired even when nitrogen is available, leading to the accumulation of nitrate (NO_3^-), amino acids, and amides in plant cells.

K^+ activates important enzymes such as nitrate reductase (NR), which is involved in nitrate assimilation, and starch synthase, which participates in carbohydrate synthesis. Through the activation of these enzymes, K^+ helps coordinate nitrogen assimilation, protein formation, and carbohydrate production.

K^+ deficiency therefore disrupts enzymatic processes involved in both nitrogen and carbohydrate metabolism, leading to an imbalance in nutrient utilization and reduced plant growth.