Applied Plant Physiology

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Tárgymutató

Applied Plant Physio

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Service Sciences
1. fejezet - 1. Principles of Applied Plant Physiology

1. 1.1. Energy source of living organisms

Living organisms need different general and special conditions to survive. General conditions are essential in all living organisms. These are: nutrients, water and optimal temperature. Nutrients provide the organism with everything it needs to grow and maintain different function.

Water is the other important material for living organisms. Living cells are 70 to 95 percent water. Much of the environment of the Earth is water based. Water can exist in all three phases (solid, liquid and gas) and can purify itself in the "water cycle." Water also has a very high specific heat, this is necessary for temperature regulation. Water is the solvent and/or medium for most of the chemical reactions that support life. Without water, these could not take place.

The optimal temperature is important because of enzymes. Temperature affects enzyme function, which affects how living cells and organisms carry out everyday processes, like making proteins and other chemical reactions. At low temperatures, enzyme function is not so efficient. At higher temperatures, the enzyme's bonds are destroyed and the enzyme is denatured, this structure loss results fails in their functions.

Special conditions are important only for a group of living organisms or/and physiological processes. These special conditions are: light, oxygen and low temperature. Several living organisms and biochemical processes being in a living cell prefer the lack of light. Presence of light can reduce, deactivate or inhibit these organisms or/and processes. The situation is the same in case of oxygen as well. Low temperature (0-5 °C) is advantageous for vernalisation, but the activity of biological pathways and enzymes are limited under this condition.

Living organisms use different sources for maintaining their life. Heterotrophs are beings, which feed themselves by eating other creatures, plants, or foods, which exist outside of themselves. All members of the kingdoms Animalia and Fungi are heterotrophs. Autotrophs are organisms, which create "food" using energy from the sun, thermal energy from the Earth, or chemical energy to feed themselves. All members of the kingdom Plantae are autotrophs. Photo-autotroph creatures are able to synthesize their own food from inorganic substances using light as an energy source. Similar to plants, photosynthetic bacteria are also photoautotrophs. Chemoautotroph organism, such as a bacterium or protozoan, that obtains its nourishment through the oxidation of inorganic chemical compounds as opposed to photosynthesis (Figure 1).

1.1. ábra - Figure 1. Summery of photo-autotroph and chemo-autotroph processes

In all of the cases, adenosine triphosphate (ATP) is the molecule, which can store the energy within cells. The 1997 Nobel Prize for Chemistry has been awarded to 3 biochemists for the study of this important biological
ATP is the nucleotide known in biochemistry as the molecular currency or coin of intracellular energy transfer. Chemically, ATP consists of adenosine and three phosphate groups. It has the empirical formula C10H16N5O13P3, and the chemical formula C10H8N4O2NH2(OH)2(PO3H)3H. ATP can be produced by various cellular processes, most typically in mitochondria by oxidative phosphorylation under the catalytic influence of ATP synthase or in the case of plants in chloroplasts by photosynthesis. The main fuels for ATP synthesis are glucose and fatty acids. ATP works by losing the endmost phosphate group when instructed to do so by an enzyme. This reaction releases a lot of energy, which the organism can then use to build proteins, contact muscles, etc. The reaction product is adenosine diphosphate (ADP), and the phosphate group either ends up as orthophosphate (HPO4) or attached to another molecule (e.g. an alcohol). Even more energy can be extracted by removing a second phosphate group to produce adenosine monophosphate (AMP). ADP and AMP can absorb energy and regain the group, thus regenerating an ATP molecule; this allows ATP to store energy like a rechargeable battery.

Some chemical reactions consume and others release energy as ATP. The terms exergonic and endergonic relate to the Gibbs free (ΔG) energy during the process. Gibbs free energy is a thermodynamic property that was defined in 1876 by Josiah Willard Gibbs to predict whether a process will occur spontaneously at constant temperature and pressure. An exergonic reaction refers to a reaction where energy is released. Reactants lose energy and ΔG is negative under constant temperature and pressure. An example of an exergonic reaction is cellular respiration. Exergonic reactions usually still require some energy to start, even though the reaction will release energy once it is complete. This extra energy is the activation energy, which a molecule temporarily stores before releasing the activation energy and some additional energy. During the endergonic reaction, energy is being absorbed as the reaction proceeds, and there is a net loss of energy in the surrounding system. Due to this consumption of energy, standard change in ΔG is a positive value under constant pressure and temperature. Endergonic reaction also requires additional energy called activation energy to start the process. During biochemical reactions, the enzymes are the bio-catalysts. A bio-catalyst can lower the activation energy barrier for the reaction. Thus, it speeds up the reaction process.

2. 1.2. Basics about enzymes

Enzymes are biological catalysts. The structure of enzymes is quite complex depending on the specific enzyme itself, but some general characteristics can be described. Primary structure of enzymes is a linear strand of all the amino acids found in the enzyme unlinked together by covalent peptide bonds. This structure determines the other structures and function of enzymes Secondary structure involves hydrogen bonds between oxygen, hydrogen, and nitrogen are found in the primary structure. This type of structure can be arranged into several configurations depending on which amino acids are present and where they are located. The most common are alpha helices and parallel/antiparallel beta sheets. Tertiary structure: the spontaneous three dimensions folding of the protein, which is due primarily to interaction between amino acid functional groups and disulfide bonds. Quaternary structure is not characteristic for all proteins, but is fairly common. This has to do with the number of globular subunits found in the enzyme and is usually maintained by the hydrophobic effect.

All of the enzymes are proteins, but sometimes they have non-protein part as well. The protein part of the enzyme is called apoenzyme and may be inactive in its original synthesized structure. The inactive form of the
apoenzyme is known as a proenzyme or zymogen. The proenzyme may contain several extra amino acids in the protein, which are removed, and allows the final specific tertiary structure to be formed before it is activated as an apoenzyme. A cofactor is a non-protein substance, which may be organic, and called a coenzyme. If the cofactor are tightly bound it is called prosthetic group like metal ion activators. The inorganic metal ions may be bound through coordinate covalent bonds. The major reason for the nutritional requirement for minerals is to supply such metal ions as Zn+2, Mg+2, Mn+2, Fe+2, Cu+2, K+1, and Na+1 for use in enzymes as cofactors. The overall enzyme contains a specific geometric shape called the active site where the reaction takes place. The molecule acted upon is called the substrate.

The basic function of an enzyme is to increase the rate of a reaction. Most cellular reactions occur about a million times faster than they would in the absence of an enzyme. The rate of a chemical reaction is affected by the total number of enzymes as well as the concentration of substrates. There is some maximum reaction rate (Vmax) when all enzyme active sites are occupied. Most enzymes act specifically with only one substrate to produce products. The most remarkable characteristic is that enzymes are regulated from a state of low activity to high activity and vice versa.

3. 1.3. Double layered fluid mosaic membrane model

Models of membranes were developed long before – from 1915 with red blood cell membrane isolates – membranes were first seen with electron microscopes in the 1950s. In 1972, S. J. Singer and G. Nicolson presented a revised model that proposed that the membrane proteins are dispersed and individually inserted into the phospholipid bilayer. In this fluid mosaic model, the hydrophilic regions of proteins and phospholipids are in maximum contact with water, and the hydrophobic regions are in a nonaqueous environment within the membrane.

The main macromolecules in membranes are lipids and proteins, but carbohydrates are also important. The most abundant lipids are phospholipids, which are amphipathic molecules: have both hydrophobic regions and hydrophilic regions. The arrangement of phospholipids and proteins in biological membranes is described by the fluid mosaic model. Membrane molecules are held in place by relatively weak hydrophobic interactions. Most of the lipids and some proteins drift laterally in the plane of the membrane, but rarely flip-flop from one phospholipid layer to the other. Many larger membrane proteins also drift within the phospholipid bilayer, although they move more slowly than the phospholipids. A membrane is a collage of different proteins embedded in the fluid matrix of the lipid bilayer. Proteins determine most of the membrane’s specific functions. The plasma membrane and the membranes of the various organelles each have unique collections of proteins. Peripheral proteins are not embedded in the lipid bilayer at all. Instead, they are loosely bound to the surface of the protein, often connected to integral proteins. Integral proteins penetrate the hydrophobic core of the lipid bilayer, often completely spanning the membrane. The hydrophobic regions embedded in the membrane’s core consist of stretches of nonpolar amino acids. Where integral proteins are in contact with the aqueous environment, they have hydrophilic regions of amino acids. The proteins of the plasma membrane have several important functions:

• Transportation: specific components into or out of cells.
• Enzymatic activity: sometimes catalyzing one of a number of steps of a metabolic pathway.
• Signal transduction: relaying hormonal messages to the cell.
• Cell-cell recognition: allowing other proteins to attach two adjacent cells together.
• Intercellular joining of adjacent cells with gap or tight junctions.
• Attachment to the cytoskeleton and extracellular matrix, maintaining cell shape and stabilizing the location of certain membrane proteins.

Membrane carbohydrates are also important for cell-cell recognition. Membrane carbohydrates are usually branched oligosaccharides with fewer than 15 sugar units. They may be covalently bonded to lipids, forming glycolipids, or more commonly to proteins, forming glycoproteins.

4. 1.4. Special organelles of plant cell
A eukaryotic cell has extensive and elaborate internal membranes, which partition the cell into compartments, organelles. These membranes also participate directly in metabolism, as many enzymes are built into membranes. The special plant organelles are: cell wall, chloroplast and vacuole.

Cell wall is a rigid wall surrounding the plasma membrane. It has complex structure, the main components of the cell wall are polysaccharides and glycoproteins. The most characteristic component found in all plant cell walls is cellulose, consists of a collection of β-1,4-ulinked glucan chains that interact with each other via hydrogen bonds to form a crystalline microfibril. Other polysaccharides are: homogalacturonan and rhamnogalacturonan I and II, xyloglucans, glucomannans, xylans, and mixed-ulinkage glucans. Plant cell walls also contain many proteins and glycoproteins, including various enzymes and structural proteins. Plant cell wall serves a variety of functions, from protecting the cell to regulating the life cycle of the plant organism. The cell wall surrounds the plasma membrane of plant cells and provides tensile strength and protection against mechanical and osmotic stress. It also allows cells to develop turgor pressure, which is the pressure of the cell contents against the cell wall.

The most important characteristic of plants is their ability to photosynthesize. This process is carried out in specialized organelles called chloroplasts. Chloroplasts are surrounded by two membranes. The outer membrane is permeable to small organic molecules, whereas the inner membrane is less permeable and studded with transport proteins. The innermost matrix of chloroplasts, called the stroma, contains metabolic enzymes and multiple copies of the chloroplast genome. Chloroplasts also have a third internal membrane called the thylakoid membrane, which is extensively folded and appears as stacks of flattened disks in electron micrographs. The thylakoids contain the light-harvesting complex, including pigments such as chlorophylls and carotenoids, as well as the electron transport chains used in photosynthesis.

Each plant cell has a large, single vacuole that stores compounds, helps in plant growth, and plays an important structural role for the plant. Matured plant cells additionally possess large, fluid-filled vesicles called vacuoles within their cytoplasm. Vacuoles typically compose about 30 percent of a cell's volume, but they can fill as much as 90 percent of the intracellular space. Plant cells use vacuoles to adjust their size and turgor pressure. Vacuoles usually account for changes in cell size when the cytoplasmic volume stays constant. Some vacuoles have specialized functions, and plant cells can have more than one type of vacuole. Vacuoles are related to lysosomes and share some functions with these structures; for instance, both contain enzymes for breaking down macromolecules. Vacuoles can also serve as storage compartments for nutrients and metabolites. For instance, proteins are stored in the vacuoles of seeds, and rubber and opium are metabolites that are stored in plant vacuoles. Plasmodesmata connected to cell walls are pores or channels between plant cell walls that allow molecules and communication signals to pass between individual plant cells.

5. 1.5. Sunlight utilization of plants

In the 1960s, photobiologist Dr. John Ott fixed the term “full-spectrum lighting” to describe light sources emitting a full spectrum of natural light, which included both ultraviolet and visible light. Ultraviolet light is a radiation with a wavelength shorter than that of visible light (10-400 nm), but longer than X-rays, Gamma rays and Cosmic rays. The visible light spectrum is actually extremely small. The infrared is above this range and the ultraviolet is just below visible light. The energy level increases as you move towards the ultraviolet and away from visible light. It means that we can find reverse ratio between the energy and the wavelength of light. Only the visible light (400-700 nm) is photosynthetically active, so provides useful energy for plants to make the food required for them to grow and flower. Plants are the only organisms able to use light to produce sugars, starches and other substances needed by them as well as by other living organisms. Flowering plants use the full spectrum of visible light, but some wavelengths are more important than others are. The right light spectrum, light intensity and light duration all work together to trigger plant flowering, growth and reproduction. The visible light spectrum emits light in red, orange, yellow, green, blue, indigo and violet colours. The most important blue wavelengths are from 430 to 450 nm. This part of the spectrum is also known as cool light. These wavelengths encourage vegetative growth through strong root growth and intense photosynthesis. Blue light is often used alone during the early phases of plant growth, such as starting seedlings, when flowering is not desired. The longer wavelengths of light are red in colour. The most important wavelengths in the red spectrum are from 640 to 680 nm. These wavelengths encourage stem growth, flowering and fruit production, and chlorophyll production. The red wavelengths are known as warm light and they are naturally more prevalent in sunlight during the shorter days of fall and winter. Different wavelengths are used for specific plant functions, but all wavelengths in this range are absorbed in varying amounts. Wavelengths in the red and blue spectrum are absorbed in greater amounts while more green and yellow light is reflected, giving the leaves their characteristic green colour. From the total amount of photosynthetically active light from the sun only 45% is available for...
plant (Figure 3). The maximum photosynthetic efficiency is 25%, but only 11% is available. Only 3-6% of the sunlight’s energy is usable by plants, losses are due to different things: conversion, reflection, etc.

1.3. ábra - Figure 3. Sunlight spectra and amount of energy utilization by plants

6. 1.6. References


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7. 1.7. Questions
1. Write the energetic equation of photo- and chemosynthesis!

2. Draw the energetic diagram of exergonic and endergonic reactions!

3. Discuss the various types of biochemical characteristics that allow biological membranes to perform the following: solute transport, macromolecular trafficking, signal transduction!

4. Analyse the structure and function correlation in the case of plant cell wall!
2. fejezet - 2. General characteristics of photosynthetic apparatus, light reaction

1. 2.1 Leaf structure of C3 and C4 plants

Leaf represents an important plant part. It is involved in vital physiological activities such as transpiration, respiration and photosynthesis. Unlike the stem and the root, the leaf is flat and hence, the anatomy of the leaf differs very much from that of stem or root. A characteristic feature of leaves is the presence of two epidermal layers, one on each surface. Both layers are composed of compactly arranged, barrel-shaped cells. Intercellular spaces are absent. A cuticle surrounds both layers. Multi-cellular hairs called trichomes are present on both layers. The cuticle plays a crucial role in plant development, and some mutants with defective cuticle exhibit morphological abnormalities, such as the fusion of organs. Stomata occur only in the lower epidermis. The ground tissue that occurs between the two epidermal layers is known as mesophyll. It is exclusively composed of chlorenchyma cells. The mesophyll is characteristically differentiated into two regions namely, an upper palisade parenchyma and a lower spongy parenchyma. Palisade parenchyma found in the upper layers of the leaf mesophyll, consisting of regularly-shaped, elongated parenchyma cells, orientated perpendicular to the leaf surface, which are active in photosynthesis. Spongy parenchyma, loosely and irregularly arranged parenchyma having numerous intercellular spaces found toward the lower surface within many leaves and consisting of irregular, lobed, or stellate cells compared to palisade parenchyma. Embedded in the mesophyll are the vascular bundles, commonly known as veins. Veins represent the vascular bundles. They are found irregularly scattered in the mesophyll due to reticulate venation. The largest and the oldest vein is found in the centre. It is known as midrib vein.

Leaves of C4 plants show two type of cells, outer mesophyll cells and inner spongy bundle sheath cells arranged in a circular manner like a necklace thus leaves of C4 plants contain two types of chloroplast, mesophyll chloroplast and bundle sheath chloroplast i.e. dimorphic chloroplast. Thus in these plants C4 pathway occurs. This anatomy of leaves in C4 plants is called Kranz anatomy.

2. 2.2. Basic reactions of photosynthesis

On a global scale, photosynthesis is the most important process on Earth. In photosynthesis, the energy that enters the chloroplasts as sunlight becomes stored as chemical energy in organic compounds. Sugar made in the chloroplasts supplies the entire plant with chemical energy and carbon skeletons to synthesize all the major organic molecules of cells. All photosynthesis occurs in the special organelles of plants called chloroplast. The photosynthesis reactions can be divided into two components (Figure 4). The light-dependent reactions (the "light" reactions) occur on the thylakoid membranes. The light-independent reactions (the "dark" reactions) take place in the stroma of chloroplasts. This dark reaction is generally called Calvin cycle. During the light plants trap sunlight energy and store it as chemical energy.

2.1. ábra - Figure 4. Location and connection of light-dependent and independent reactions in the chloroplast
3. 2.3. Photosynthetic pigments

Pigments are compounds, which reflect only certain wavelengths of visible light. Flowers, corals, and even animal skin contain pigments which give them their colors. Chlorophyll and carotenoids are both a group of photosynthetic pigments that are involved in photosynthesis. Both chlorophyll and carotenoids are responsible for harvesting light, absorbing photons and transferring the excitation energy to the photosynthetic reaction centre. Only chlorophyll, however, functions within the reaction centre to perform charge separation across the cell membrane. Chlorophyll was first identified in 1818 by Pierre Joseph Pelletier and Joseph Bienaimé Caventou. Chlorophyll is well-known for its green appearance and for being the most abundant photosynthetic pigment on Earth. Since its original discovery, dozens of types of chlorophyll molecules have been discovered. Molecularly, they are all cyclic tetrapyrroles and usually contain a central magnesium ion. This porphyrin ring makes stable the molecule and electrons are free to migrate. Chlorophyll’s chemical structure has the potential to gain or lose electrons easily, which is what allows it to absorb photons and transfer the excitation energy to and within the photosynthetic reaction centre. Chlorophyll and carotenoids are both light-harvesting pigments, but chlorophyll is the most abundant and the most critical for photosynthesis. The different types of chlorophylls, working in combination, are able to absorb light over much of the photosynthetic spectrum, from 330-1,050 nm with one exception is what is called the “green gap,” around 500 nm. Accessory pigments are required to fill this absorption gap. A second limitation of chlorophylls arises from the characteristic that makes them such powerful pigments in the photosynthetic system: their ability to maintain long-lived excited states. That ability, however, also leads to a tendency to generate toxic reactive oxygen species. Again, accessory pigments, carotenoids in particular, are able to help solve this problem.

Carotenoids are chromophores that are usually red, orange or yellow in colour. The most well-known carotenoid is probably β-carotene, which gives carrots their orange colour. These compounds are composed of two small six-carbon rings connected by a "chain" of carbon atoms. As a result, they do not dissolve in water, and must be attached to membranes within the cell. Carotenoids cannot transfer sunlight energy directly to the photosynthetic pathway, but must pass their absorbed energy to chlorophyll. For this reason, they are called accessory pigments. Carotenoids have two main functions: harvesting light energy for photosynthesis and protecting chlorophyll from light damage. For their primary function, carotenoids absorb light energy from photons. Along with biliproteins, they help absorb energy in the “green gap” near 500 nanometers. They transfer the excitation energy directly to chlorophyll molecules, which then transfer the energy to reaction centres and into the photosynthetic pathway. Carotenoids and chlorophyll and carotenoids together make up the light-harvesting antenna within cells. The other important function of carotenoids is protecting chlorophyll and the surrounding cell from light damage. During the photosynthetic light- reaction chlorophylls often generate toxic reactive oxygen species, which cause diverse cellular damage, and they are particularly prone to generating such free radicals under high light conditions. Carotenoids are able to absorb excess light, diverting it from chlorophyll and have special antioxidant skills. Unlike chlorophyll, carotenoids can harmlessly convert excess excitation energy to heat.
4. 2.4. Energy transformation in photosynthesis

Photosynthesis can be defined as the physico-chemical process by which photosynthetic organisms use light energy to drive the synthesis of organic compounds. The photosynthetic process depends on a set of complex protein molecules that are located in and around a highly organized membrane of chloroplast. Through a series of energy transducing reactions, the photosynthetic machinery transforms light energy into a stable form.

2.2. ábra - Figure 5. Energy transformation from light energy to chemical bond energy during the photosynthetic light reaction Withmarsh, J., Govindje, N. (1999)

The light reactions convert sunlight energy into several forms. The theoretical minimum quantum requirement for photosynthesis is 8 quanta for each molecule of oxygen evolved. When photosynthetic pigments absorb light energy, electron gains energy and is excited in the antenna system. Photosynthetic pigments form antenna systems, which are anchored to proteins within the photosynthetic membrane and serve a specialized protein complex known as a reaction centre. The electronic excited state is transferred over the antenna molecules as an excitation. Some excitations are converted back into photons and emitted as fluorescence, some are converted to heat, and some are trapped by a reaction centre protein. Excitations trapped by a reaction centre provide the energy for the primary photochemical reaction of photosynthesis - the transfer of an electron from a donor molecule to an acceptor molecule. The excited electron is transferred to another molecule called a primary electron acceptor. The chlorophyll molecule is oxidized and has a positive charge. Photoactivation of chlorophyll results in the splitting of water molecules and the transfer of energy to ATP and reduced nicotinamide adenine dinucleotide phosphate (NADP). Photosynthesis is a two-stage process. The light dependent reactions, a light-dependent series of reactions, which occur in the grana thylakoids, and require the direct energy of light to make energy-carrier molecules that, are used in the second process. Light energy is
2. General characteristics of photosynthetic apparatus, light reaction

trapped by chlorophyll to make ATP (photophosphorylation), at the same time water is split into oxygen, hydrogen ions and free electrons:

\[ 2\text{H}_2\text{O} \rightarrow 4\text{H}^+ + \text{O}_2 + 4\text{e}^- \] (photolysis)

The electrons then react with a carrier molecule nicotinamide adenine dinucleotide phosphate (NADP), changing it from its oxidised state (NADP\(^+\)) to its reduced state (NADPH):

\[ \text{NADP}^+ + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{NADPH} + \text{H}^+ \]

When light energy is absorbed by a chlorophyll molecule its electrons gain energy called photoexcitation and move to higher energy levels in the molecule. Photoionisation means, that sufficient energy ionises the molecule, with the electron being 'freed' leaving a positively charged chlorophyll ion. In whole chloroplasts each chlorophyll molecule is associated with an electron acceptor and an electron donor. Two electrons from a photoionised chlorophyll molecule are transferred to the electron acceptor. The positively charged chlorophyll ion then takes a pair of electrons from a neighbouring electron donor such as water. An electron transfer system (a series of chemical reactions) carries the two electrons to and fro across the thylakoid membrane. The energy to drive these processes comes from two photosystems, Photosystem II (PSII) with the P680 reaction centre and Photosystem I (PSI) with the P700 reaction centre. The flow of photosynthetic reactions start with PSII but it is named II because it was the second to be discovered. PSII and PSI work concurrently but in series. In the light photosystem II feeds electrons to photosystem I. The electrons are transferred from photosystem II to the photosystem I by intermediate carriers. The net reaction is the transfer of electrons from a water molecule to NADP\(^+\), producing the reduced form, NADPH. In the photosynthetic process, much of the energy initially provided by light energy is stored as redox free energy (a form of chemical free energy) in NADPH, to be used later in the reduction of carbon. The energy changes accompanying the two sets of changes make a Z shape when drawn out. This is why the electron transfer process is sometimes called the Z scheme. Key to the scheme is that sufficient energy is released during electron transfer to enable ATP to be made from ADP and phosphate. The electron transfer reactions concentrate protons inside the membrane vesicle and create an electric field across the photosynthetic membrane. In this process the electron transfer reactions convert redox free energy into an electrochemical potential of protons. The energy stored in the proton electrochemical potential is used by a membrane bound protein complex (ATP-Synthase) to covalently attach a phosphate group to adenosine diphosphate (ADP), forming adenosine triphosphate (ATP). Protons pass through the ATP-Synthase protein complex that transforms electrochemical free energy into a type of chemical free energy known as phosphate group-transfer potential. The energy stored in ATP can be transferred to another molecule by transferring the phosphate group. The NADPH and ATP formed by the light reactions provide the energy for the dark reactions of photosynthesis, known as the Calvin cycle or the photosynthetic carbon reduction cycle. The reduction of atmospheric CO\(_2\) to carbohydrate occurs in the aqueous phase of the chloroplast and involves a series of enzymatic reactions.

5. 2.5. Non-cycling electron-transport

Non-cycling electron-transport takes place in grana thylakoids of chloroplast. During this process both adenosine triphosphate and NADPH are produced. Electron transport from water to NADP\(^+\) requires three membrane bound protein complexes operating in series - photosystem II, the cytochrome b6f complex and photosystem I.

In photosystem II:

- photoionisation of chlorophyll transfers excited electrons to an electron acceptor
- photolysis of water (an electron donor) produces oxygen molecules, hydrogen ions and electrons, and the latter are transferred to the positively-charged chlorophyll
- the electron acceptor passes the electrons to the electron transport chain; the final acceptor is photosystem PSI. Electrons are transferred between large protein complexes by small mobile molecules, plastoquinone. Plastoquinone transfers electrons from the photosystem II reaction centre to the cytochrome b6f complex and carries protons across the photosynthetic membrane. The cytochrome b6f complex is a membrane bound protein complex that contains four electron carriers, three cytochromes and a FeS centre. The crystal structure has been solved for cytochrome f from turnip. Electron transfer from the cytochrome b6f complex to photosystem I is mediated by a small Cu-protein, plastocyanin (PC). Plastocyanin is water soluble and operates in the inner water space of the photosynthetic membrane. Electron transfer from photosystem I to
NADP+ requires ferredoxin, a small FeS protein, and ferredoxin-NADP oxidoreductase, a peripheral flavoprotein that operates on the outer surface of the photosynthetic membrane. Ferredoxin and NADP+ are water soluble and are found in the outer aqueous phase

- further absorbed light energy increases the energy of the electrons, sufficient for the reduction of NADP+ to NADPH

Electrons passing through the transport chain provide energy to pump H+ ions from the stroma, across the thylakoid membrane into the thylakoid compartment. H+ ions are more concentrated in the thylakoid compartment than in the stroma. We say there is an electrochemical gradient. H+ ions diffuse from the high to the low regions of concentration. This drives the production of ATP. The conversion of proton electrochemical energy into chemical free energy is accomplished by a single protein complex known as ATP synthase. This enzyme catalyzes a phosphorylation reaction, which is the formation of ATP by the addition of inorganic phosphate to ADP. The reaction is energetically uphill (ΔG = +32 kJ/mol) and is driven by proton transfer through the ATP synthase protein. The molecular processes that couple proton transfer through the protein to the chemical addition of phosphate to ADP are poorly understood. It is known that phosphorylation can be driven by a pH gradient, a transmembrane electric field, or a combination of the two. Experiments indicate that three protons must pass through the ATP synthase complex for the synthesis of one molecule of ATP.

6. 2.6. Cycling electron-flow in photosynthesis

The net effect of non-cyclic phosphorylation is to pass electrons from water to NADP. Energy released enables the production of ATP. However, much more ATP is needed to drive the light-independent reactions. This extra energy is obtained from cyclic phosphorylation which takes place in stroma thylakoids. This involves only Photosystem I, which generates excited electrons. These are transferred to the electron transport chain between PSII and PSI, rather than to NADP+ and so no NADPH is formed. The cyclic electron transport pathway begins after the PS-I pigment complex absorbs solar energy. They transfer their energy to PS-I reaction centre - P700. The outer valence electron of excited P700 is raised to higher energy level which is captured by the primary acceptor of PS-I. The primary acceptor then transfers electron to ferredoxin. Reduced ferredoxin, unable to reduce NADP+ returns the electron to PS-I via cyt b6, PQ, cyt f and PC. The cycle is completed by electrons being transported back to PSI by the electron transport system. Cyclic electron transport around the PSI reaction centre is generally accepted to generate a pH gradient across the thylakoid membrane. This ΔpH might serve a number of functions; however, the most obvious to consider in the context of CET are the generation of ATP and the regulation of light harvesting, via the process of non-photochemical quenching. Most cycling electron transport will be occurring under conditions where light absorption and linear electron transport tend to be in excess of the capacity for carbon fixation by the Calvin cycle. Under such conditions, the flow of electrons through the linear chain is down-regulated, via regulation of the cytochrome b6f complex. This leads to a situation where the plastoquinone pool is largely reduced and the components of the electron transfer chain after plastoquinol oxidation (P700, cyt f, plastocyanin) oxidized. If cyclic electron flow is to occur under such conditions, it must be able to inject electrons into the plastoquinone pool, in competition with PSII.

In C4 plants, cycling electron transport is presumed to be a phenomenon associated with bundle sheath cells, where PSII is largely absent and so cycling electron transport can be the only significant form of photosynthetic electron transport. In these cells, malate exported from the mesophyll is broken down generating CO2 and NADPH.

7. 2.7. References


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2. General characteristics of photosynthetic apparatus, light reaction


8. 2.8. Questions

1. What are the products of light reaction?

2. Compare cyclic and non-cyclic electron transport!

3. What is the role of electron transport in the photosynthetic light reaction?
3. fejezet - 3. Physiological and anatomical aspects of dark reaction in photosynthesis

1. 3.1. Dark reaction, Calvin-Benson-Bassham cycle

Light dependent reaction of photosynthesis is responsible for creating NADPH and ATP and the Calvin-Benson-Bassham cycle uses these high energy molecules to drive the production of glyceraldehyde-3-phosphate and hexose sugars. The Calvin cycle/Calvin–Benson–Bassham cycle/reductive pentose phosphate cycle/C3 cycle/Light independent cycle – same cycle with different names - is a series of biochemical redox reactions that take place in the stroma of chloroplasts in photosynthetic organisms. The cycle was discovered in 1950 by Calvin, Bassham and Benson. Although the Calvin cycle has been given the nick name the “dark reaction” the enzymes involved are activated by light. Light stimulates changes in pH in the different regions of the plant cell which then in turn create a better environment for the Calvin-cycle enzymes. The enzymes in the cycle are very similar to other enzymes found in other metabolic pathways with the exception that they are found in the stoma instead of in the cytoplasm like in glycolysis. All plants and algae remove CO2 from the environment and reduce it to carbohydrate by the Calvin cycle. The process is a sequence of biochemical reactions that reduce carbon and rearrange bonds to produce carbohydrate from CO2 molecules.

2. 3.2. Phases of Calvin cycle

Overall, thirteen enzymes are required to catalyze the reactions in the Calvin cycle. The energy conversion efficiency of the Calvin cycle is approximately 90%. The reactions do not involve energy transduction, but rather the rearrangement of chemical energy. Each molecule of CO2 reduced to a sugar [CH2O]n requires 2 molecules of NADPH and 3 molecules of ATP. There are three phases of the cycle (Figure 6).

3.1. ábra - Figure 6. The abbreviated version of the Calvin cycle (Raven et al., 2005)
The first step in the first phase is the addition of CO2 to a five-carbon compound, ribulose 1,5-bisphosphate. The six-carbon compound is split, giving two molecules of a three-carbon compound, 3-phosphoglycerate. This key reaction is catalyzed by RuBisCO (ribulose bisphosphate carboxylase oxygenase) using its carboxylase function. This enzyme catalyses the carboxylation of ribulose-1,5-bisphosphate in a two step reaction. Ribulose-1,5-bisphosphate must first be phosphorolated by the enzyme Phosphoribulose kinase. The outcome of this carboxylation are two molecules of 3-Phosphoglycerate. The carboxylation reaction is energetically downhill. This stage is very similar to the isomerization phase of Pentose-Phosphate Pathway.

The second phase of Calvin cycle very closely resembles part of gluconeogenesis. The 3-Phosphoglycerate is then phosphorolated with Phosphoglycerate kinase to yield 1,3-Bisphosphoglycerate. Next 1,3-Bisphosphoglycerate is reduced by NADPH to yield NADP+ and Glyceraldehyde-3-phosphate with Glyceraldehyde-3-phosphate dehydrogenase. One of every six Glyceraldehyde-3-phosphate molecules is exported into the cytoplasm to be used in the synthesis of glucose and other metabolic processes. The main energy input in the Calvin cycle is the phosphorylation by ATP and subsequent reduction by NADPH of the initial three-carbon compound forming a three-carbon sugar, triosephosphate. Some of the triosephosphate is exported from the chloroplast and provides the building block for synthesizing more complex molecules.

During the regeneration phase (Phase 3) Glyceraldehyde-3-phosphate is reversibly converted to Dihydroxyacetone phosphate by Triose phosphate isomerase. Next Dihydroxyacetone is converted into fructose-6-phosphate (F-6-P) by Aldolase and Fructose bisphosphatase. Aldolase condenses the two DHAP molecules to form Fructose-1,6-bisphosphate. Because of its high (-)G the transformation of Fructose-1,6-bisphosphate to Fructose-6-phosphate is thought to be the rate limiting step of the CBB cycle. F-6-P can then be converted into glucose via two enzymatic steps with the help of Phosphoglucoisomerase and glucose-6-Phosphatase. Dihydroxyacetone can also go on to condense with Erythrose-4-phosphate to form Sedoheptulose-1,7-bisphosphate (SBP). This reaction is also catalyzed by Aldolase. SBP is then de-phosphorolated by Sedoheptulose bisphosphatase to yield Sedoheptulose-7-phosphate (S7P). After several rearrangement reactions
3. Physiological and anatomical aspects of dark reaction in photosynthesis

utilizing Transketolaseand Transaldolaseenzymes, Xylulose-5-Phosphate (X5P) and Ribose-5-phosphate (R5P) are synthesized. Finally, X5P and R5P are isomerised by Phosphopentose epimerase and Phosphopentose isomerase to yield Ribulose-5-phosphate which can then be put back into the cycle. In this regeneration phase the Calvin cycle uses some of the triosephosphate molecules to synthesize the energy rich ribulose 1,5-bisphosphate needed for the initial carboxylation reaction. This reaction requires the input of energy in the form of one ATP and really resembles the rearrangement phase of Pentose-Phosphate-Pathway.

3. 3.3 Starch storage of chloroplast

The glucose is stored mainly in the form of starch granules, in plastids such as chloroplasts and especially amyloplasts. Glucose molecules are bound in starch by the easily hydrolyzed alpha bonds. Plants produce starch by first converting glucose 1-phosphate to ADP-glucose using the enzyme glucose-1-phosphate adenylyltransferase. This step requires energy in the form of ATP. The enzyme starch synthase then adds the ADP-glucose via a 1,4-alpha glycosidic bond to a growing chain of glucose residues, liberating ADP and creating amyllose. Starch branching enzyme introduces 1,6-alpha glycosidic bonds between these chains, creating the branched amylopectin. The starch debranching enzyme isoamylase removes some of these branches. Several isoforms of these enzymes exist, leading to a highly complex synthesis process. Strach plays an important role in the day-to-day carbohydrate metabolism of the leaf, and its biosynthesis and degradation represent major fluxes in plant metabolism. The mechanism has diurnal cycles control. Nowadays evidence emerged that a dual-specificity protein phosphatase (DSP4) binds to starch granules during the day and dissociates at night. Disruption of the DSP4 gene resulted in a dramatic increase in the level of starch in mutant Arabidopsis plants. Moreover, although composition was apparently unchanged, the morphology of the starch granule was significantly altered compared to the wild type counterpart. Two regulatory factors unlinked to light (i.e., pH and redox status) changed both the activity and the starch-binding capacity of DSP4. Glucose molecules are bound in starch by the easily hydrolyzed alpha bonds. Plants produce starch by first converting glucose 1-phosphate to ADP-glucose using the enzyme glucose-1-phosphate adenylyltransferase. This step requires energy in the form of ATP. The enzyme starch synthase then adds the ADP-glucose via a 1,4-alpha glycosidic bond to a growing chain of glucose residues, liberating ADP and creating amyllose. Starch branching enzyme introduces 1,6-alpha glycosidic bonds between these chains, creating the branched amylopectin. The starch debranching enzyme isoamylase removes some of these branches. Several isoforms of these enzymes exist, leading to a highly complex synthesis process.

4. 3.4 RuBisCO: structure and function

The 3-dimensional structure has been determined by X-ray analysis for RuBisCO isolated from tobacco (Schreuder et al. 1993). RuBisCO is a bifunctional enzyme that, in addition to binding CO2 to ribulose bisphosphate, can also bind O2. This oxygenation reaction produces the 3-phosphoglycerate that is used in the Calvin cycle and a two-carbon compound (2-phosphoglycolate) that is not useful for the plant. In response, a complicated set of photorespiration are initiated that serve to recover reduced carbon and to remove phosphoglycolate. Some plants have evolved specialized structures and biochemical pathways that concentrate CO2 near RuBisCO. These pathways (C4 and CAM), serve to decrease the fraction of oxygenation reactions. RuBisCO is the most abundant enzyme on Earth. The whole enzyme can be devided to units: 8 large catalytic subunits (L, 477 amino acid residues) and 8 small subunits (S, 123 amino acid residues). Large subunits within RuBisCO are arranged as antiparallel dimers, with the N-terminal domain of one monomer adjacent to the C-terminal domain of the other monomer. Each active site is at an interface between monomers within an L2 dimer, explaining the minimal requirement for a dimeric structure. The substrate binding site is at the mouth of an ₪β-barrel domain of the large subunit. Most active site residues are polar, including some charged amino acids (e.g., Thr, Asn, Glu, Lys). The active RuBP Carboxylase includes a carbamate group, that binds an essential Mg2+ at the active site. The reversible formation of a carbamate by reaction of CO2 with the amino group of a lysine residue in the catalytic site and its stabilization by Mg2+ is a basic mechanism underlying the control of RuBisCO activity. HCO3⁻ that reacts to form the carbamate group is distinct from CO2 that binds to RuBP Carboxylase as substrate. The active site Mg2+ bridges between oxygen atoms of the carbamate and the substrate CO2.

5. 3.5. Photorespiration

Photorespiration is a process at least partially opposing photosynthesis. The mechanistic basis of photorespiration was found in the dual nature of ribulose bisphosphate (RuBP) carboxylase/oxygenase. Which
one predominates depends on the relative concentrations of O2 and CO2 with high CO2, low O2 favouring the carboxylase action, high O2, low CO2 favoring the oxygenase action. The light reactions of photosynthesis liberate oxygen and more oxygen dissolves in the cytosol of the cell at higher temperatures. Therefore, high light intensities and high temperatures favour the second reaction. The photosynthetic carboxylation reaction yields two molecules of 3-phosphoglycerate (3PGA), which enter the C3 carbon reduction or Calvin cycle, whereas oxygenation, which competitively inhibits carboxylation, produces one molecule of 3PGA and one molecule of the 2-carbon compound phosphoglycolate. The photorespiratory pathway is the conversion of phosphoglycolate to CO2 and 3PGA, a complex series of reactions that takes place across three separate subcellular compartments; chloroplasts, peroxisomes, and mitochondria. In mitochondria carbon dioxide develops as a product of the serine synthesis. Serine is synthesized under light exposure and oxygen uptake from two glycerines. During the synthesis of glyoxalate from glycolate is the heavy cytotoxin hydrogen peroxide produced that is immediately afterwards broken down by the enzyme catalase. Catalase is an enzyme specific for peroxysomes.

Photorespiration results in the loss of up to 25% of the carbon that is fixed during photosynthetic carbon assimilation. Despite the net loss of carbon, the photorespiratory cycle allows for recovery into the C3 carbon assimilation cycle of three moles of carbon (as 3PGA) for every one that is respired as CO2.

6. 3.6. Main characteristics of C4 and CAM photosynthetic pathways

C4 plants – maize, sugarcane, sorghum – have developed adaptations which minimize the losses to photorespiration. They all use a supplementary method of CO2 uptake which forms a 4-carbon molecule instead of the two 3-carbon molecules of the Calvin-cycle. They have structural changes in their leaf anatomy as we discussed in chapter 2. The primary and secondary CO2 fixations are separated in different parts of the leaf and RuBisCO sequestered where the CO2 level is high; the O2 level low. During the primary CO2 fixation phosphoenolpyruvate carboxylase enzyme produce 4-carbon compound oxaloacetic acid (C4). Oxaloacetic acid is converted into malic acid or aspartic acid, which is transported into a bundle sheath cell. Bundle sheath cells are deep in the leaf so atmospheric oxygen cannot diffuse easily to them and the number of grana thylakoids is really low. Stroma thylakoids contain only PSI, the oxygen level remains reduced. Oxidative decarboxilation process occurs in the bundle sheath cells producing CO2 and NADPH, which are consumed by Calvin cycle in the stroma of bundle sheath cell chloroplasts.

In the context of C4 pathway, three biochemical subtypes have been defined that differ in the subcellular localization and type of C4 acid decarboxylase used in the bundle sheath cells. The first to be discovered was the NADP-malic enzyme (ME) type, in which the decarboxylation step is performed in bundle sheath cell chloroplasts by NADP-dependent ME. By contrast, the NAD-ME and phosphoenolpyruvate carboxykinase (PEP-CK) subtypes both move aspartic and alanin between mesophyll and bundle sheath cells. Aspartic acid is converted to either malate or oxalacetic acid, and then malate is decarboxylated by NAD-ME in the bundle sheath cell mitochondria or oxalacetic acid is decarboxylated by PEP-CK in the BS cell cytoplasm. The NAD-ME and PEP-CK pathways have higher energy requirements than the NADP-ME pathway, and both have more intracellular transport steps.

3.2. ábra - Figure 7. Brief review on C4 photosynthesis (based on Langdale, 2011)
CAM plants - Crassulacean Acid Metabolism – are also C4 plants but instead of segregating the C4 and C3 pathways in different parts of the leaf, they separate them in time. The ratio of water loss to CO2 uptake is much lower in CAM plants than it is in either C3 or C4 plants. This is because stomata are open only at night when temperatures are lower and humidities higher than daytime conditions, both of which contribute to a lower transpiration rate. This photosynthetic adaptation is important because of surviving high daily temperature, high sunlight and the lack of water. CAM plants take in CO2 through their open stomata at night because a preferable temperature. The CO2 fix on phosphoenolpyruvic acid and forms oxaloacetic acid. This is converted to malic acid that accumulates during the night in the central vacuole of the cells. The stomata are closed in the morning. The accumulated malic acid leaves the vacuole and is broken down to release CO2, which is taken up into the Calvin cycle.

3.1. táblázat - Table 1. Comparison of main characteristics of different photosynthetic pathways

<table>
<thead>
<tr>
<th>Feature</th>
<th>C3</th>
<th>C4</th>
<th>CAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf structure</td>
<td>Mesophyll cells with chloroplast</td>
<td>Bundle sheath cells having chloroplasts without grana</td>
<td>Large vacuoles in mesophyll cells</td>
</tr>
<tr>
<td>Enzyme to fix CO2</td>
<td>RuBiCo</td>
<td>PEPcarboxylase</td>
<td>PEPcarboxylase</td>
</tr>
<tr>
<td>Primary acceptor</td>
<td>of RUBP: 6C compound</td>
<td>PEP: 3C compound</td>
<td>PEP: 3C compound</td>
</tr>
</tbody>
</table>
3. Physiological and anatomical aspects of dark reaction in photosynthesis

<table>
<thead>
<tr>
<th>CO2</th>
<th>First stable product</th>
<th>oxaloacetic acid</th>
<th>oxaloacetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficiency</td>
<td>lower/photo-respiratory losses</td>
<td>higher/photo-respiratory losses</td>
<td>higher/photo-respiratory losses</td>
</tr>
<tr>
<td>Optimum temperature</td>
<td>15-25°C</td>
<td>30-40°C</td>
<td>35°C</td>
</tr>
</tbody>
</table>

7. 3.7. References


http://www.bio.miami.edu/~cmallery/150/phts/c8.10x21.overview.jpg


8. 3.8. Questions

1. Outline the structure and function of RuBisCO!

2. Give comparison between the following: C3 and C4 pathways, anatomy of leaf in C3 and C4 plants!

3. What are the advantages and disadvantages of photorespiration?

4. Describe the phases of Calvin cycle!
4. fejezet - 4. Response of photosynthesis to light and different availability of water

1. 4.1. Properties of light

Light, usually referred to as visible light is electromagnetic radiation that is visible to the human eye. Some properties of light important for plants are listed below:

1. quantity: the intensity or amount of light (mol m$^{-2}$s$^{-1}$)
2. quality: the wavelength or colour of light (nm)
3. duration: determines the total amount of light energy received
4. periodicity: the day length, or length of light in a 24 hour cycle, regardless of quantity.

Light can be affected as follows:

- absorbed: when radiant energy (such as light) is absorbed it is converted primarily to heat energy
- re-radiation: heat energy is converted to radiant energy as long wavelengths in the infrared (IR) region of the spectrum
- transmitted: when light passes through an object unaffected, such as glass.
- reflected or scattered: when light is "bounced off" an object, such as a solid colored surface.

Light intensity, light quantity refers to the total amount of light that plants receive and one of the most important abiotic factors influencing plant life. It is also described as the degree of brightness that a plant is exposed to. This parameter can be measured and characterised in different ways.

- photometer or common light meter: measures amount of luminance. Expressed as: foot-candle (ft-c): 1 lumen per square foot and lux: 1 lumen per square meter 1 foot-candle = 10.76 lux
- quantum sensor: measures actual light intensity or light energy in the 400-700 nm wavelength band photosynthetically active radiation (PAR): light intensity in the 400-700 nm wavelength band that is used by PLANTS in photosynthesis. Expressed as: a) microEinstein per second per square meter (mEs-1m$^{-2}$) (400-700 nm) b) watts per square meter (Wm$^{-2}$) (400-700 nm)

Several factors affect light intensity. The intensity of light can change with the time of the day, season, geographic location, distance from the equator, and weather. It gradually increases from sunrise to the middle of the day and then gradually decreases toward sunset; it is high during summer, moderate in spring and fall, and low during winter time. Maximum intensity occurs at the equator, and gradually decreases with increasing distance from the equator to the south and north poles. Light intensity is also affected by dust particles and atmospheric water vapour, slope of the land, and elevation. Depending on the particular time of the year, the sun-to-earth distance varies and this causes a slight variation in the amount of light and heat that the earth receives. Leaves on a single plant differ for light that they receive. The amount of light incident on a leaf decreases as sunlight passes downward through the canopy. Leaves on the upper part of the canopy tend to shade and reflect light away from the lower leaves. Plants with somewhat vertical leaves allow more downward passage of light and tolerate high population planting than plants with drooping leaves.

The more sunlight a plant receives, to a degree, the higher the photosynthetic rate will be.

2. 4.2. Light response curves of photosynthesis
4. Response of photosynthesis to light and different availability of water

Light is a critical resource for plants that can limit growth and reproduction. When photosynthesis rates are plotted against light intensity, the result is a photosynthesis light response curve (Figure 8). In the dark, there is no photosynthetic carbon assimilation, and instead CO2 is given off by the plant because of mitochondrial respiration. By convention, CO2 assimilation is negative in this part of the light-response curve. As the photon flux increases, photosynthetic CO2 assimilation increases linearly until it equals CO2 release by mitochondrial respiration. The point at which photosynthetic CO2 uptake exactly balances CO2 release is called the light compensation point. Increasing light above the light compensation point proportionally increases photosynthesis, indicating that photosynthesis is limited by the rate of electron transport, which in turn is limited by the amount of available light. This portion of the curve is referred to as light-limited. Further increases in photosynthesis are eventually limited by the carboxylation capacity of RubisCO or the metabolism of triose phosphates. This part of the curve is referred to as CO2-limited.

4.1. ábra - Figure 8. Generalized photosynthetic light response curve of C3 plants

![Generalized Photosynthetic Light Response Curve](image)

Different plants, even different leaves on the same plant show differences in the shape of their light response curves. The curve reveals characteristics of the underlying photosynthesis processes including the light-dependent and light-independent reactions, the efficiency at which light is utilized by photosynthesis, and even the rate of O2 uptake.

Plants capable of C4 photosynthesis carry on a more efficient form of photosynthesis. There are two crucial differences between the light response curve of C3 and C4 plants. For C4 plants, the light saturation point is higher and the light compensation point is lower than for C3 plants. Both of these characteristics relate to the ability of C4 plants possess to increase the amount of CO2 available to the Calvin-Benson cycle.

In general, photosynthesis can function without harm between 0 and 30 °C in cold-adapted plants that are active in winter and early spring, or grow at high altitude and latitude. Photosynthesis shows an optimum temperature that roughly corresponds to the middle of the non-harmful range, and drops off with increasing slope as
4. Response of photosynthesis to light and different availability of water

Temperatures rise above the thermal optimum. At lower temperatures, the quantum yield of C3 plants is higher than that of C4 plants, indicating that photosynthesis in C3 plants is more efficient at lower temperatures. After the saturation point, photosynthesis is CO2 limited reflecting the inability of the Calvin cycle enzymes to keep pace with the absorbed light energy that is producing ATP and NADPH. Light saturation levels for shade plants are substantially lower than those for sun plants. These levels usually reflect the maximum photon flux to which the leaf was exposed during growth.

If the light intensity is too high mainly parallel with other unfavourable abiotic conditions, such as high temperature, photodamages may have been occurred in photosynthetic apparatus.

3. 4.3. Light in excess

Plants have evolved functional and structural features that reduce the excess light load on leaves during high sunlight periods, especially when transpiration are reduced because of water stress. These features often involve changes in the leaf orientation relative to the incoming sunlight. Diaphototropism can serve to maximize carbon gain by increasing incident photon flux density. Paraphototropic leaves track the sun but at the same time can reduce incident light levels by folding leaflets together so that the leaf lamina becomes nearly parallel to the sun’s rays. Under low light conditions, chloroplasts accumulate along the cell walls that are perpendicular to the incident light. Under high light conditions, they accumulate along the walls that are parallel to the incident light. These are the regions of plant leaf cells where internal fluence rates of light are the highest and lowest, respectively and it is believed likely that the light-induced chloroplast movements serve an adaptive function. We can list here plant wilting as well, whereby a leaf droops to a vertical orientation, again effectively reducing the incident heat load and reducing transpiration and incident light levels.

When exposed to excess light, leaves must dissipate the surplus absorbed light energy so that it does not harm the photosynthetic apparatus (Figure 9). Under unfavourable conditions, when the stomata are closed, high energy electrons produced by the non-cyclic electron transport can reach the molecular oxygen, which easily becomes reactive superoxide. There are several routes for energy dissipation involving nonphotochemical quenching, which is the quenching of chlorophyll fluorescence by mechanisms other than photochemistry.

4.2. ábra - Figure 9. Different way of absorbed light

![Figure 9](image_url)

The most important example involves the transfer of absorbed light energy away from electron transport toward heat production. Although the molecular mechanisms are not yet fully understood, the xanthophyll cycle appears to be an important way for dissipation of excess light energy. Xanthophyll cycle consists of three carotenoids (violaxanthin, antheraxanthin, zeaxanthin) connects to PSII in thylakoid membrane (Figure 10). Under high light, violaxanthin is converted to antheraxanthin and then to zeaxanthin. Zeaxanthin is the most effective of the three xanthophylls in heat dissipation, and antheraxanthin is only half as effective. Whereas the levels of antheraxanthin remain relatively constant throughout the day in most of the investigated species, the zeaxanthin content increases at high irradiances and decreases at low irradiances. In leaves, growing under full sunlight a substantial amount of excess light energy absorbed by the thylakoid membranes can be dissipated as heat, thus preventing damage to the photosynthetic machinery of the chloroplast.

4.3. ábra - Figure 10. Connection of xanthophyll cycle to LHCII complex in the thylakoid membrane of chloroplast

![Figure 10](image_url)
4. Response of photosynthesis to light and different availability of water

Leaves growing in full sunlight contain a substantially larger xanthophyll pool than do shade leaves, so they can dissipate higher amounts of excess light energy. In addition, smaller but really active xanthophyll cycle pool is also beneficial for plants under high light intensity.

When leaves are exposed to more light than they can utilize the reaction center of PSII is inactivated. This phenomenon is called photoinhibition. Under moderate excess light dynamic photoinhibition is caused by the diversion of absorbed light energy toward heat dissipation. The decrease in quantum efficiency is often temporary, and quantum efficiency can return to its initial higher value when photon flux decreases below saturation levels. Chronic photoinhibition results from exposure to high levels of excess light that damage the photosynthetic system and decrease both quantum efficiency and maximum photosynthetic rate. Chronic photoinhibition is associated with damage and replacement of the D1 protein from the reaction centre of PSII.

4. 4.4. Effect of light quality on plants

The quality of light can affect plant life in different levels and ways.

• photosynthesis: light dependent reaction and the level of stomatal open-close movement

• phototropism: plants bend towards areas of higher light intensity

• temperature: high light intensity increases temperature due to absorption of radiation and greenhouse effect

• transpiration: more intensive under high light intensity due to heat buildup. BUT transpiration may decrease in the case of stomata closure

• sun/shade plants: different leaf structure, sun grown leaf has thicker blade due to thicker palisade parenchyma layer compared to shade grown leaf, which has higher proportion of spongy mesophyll). Leaves growing in shade have larger size, than sun adapted ones. Finally, the optimum light intensity also shows differences, shade plants prefer low light intensity for maximum photosynthesis
4. Response of photosynthesis to light and different availability of water

- photooxidation: negative effect of excess light means destruction of photosynthetic system
- etiolation: elongated shoot with tiny leaves, pale green to yellowish growth due to low light intensity
- blanching: lack of color development due to exclusion of light (cauliflower, asparagus and celery)
- light acclimatization: short time conditioning of plants to low light intensity interior environments.

5. 4.5. Effect of light quantity on plants

- photosynthesis: chlorophylls absorb predominantly blue and orange-red light, while green-yellow is transmitted and reflected
- growth responses: due to effect on photosynthesis in the plant canopy shade part is rich in green-yellow and far red, poor in blue and orange-red light
- pigments: anthocyanins: blue, red and purple in color. Carotenoids: orange and yellow in color and absorb 450-500 nm (blue and green). Carotenoids helper and defender group of photosynthetic pigments. Phytochromes are light-receptor protein kinases that are regulated by red and far-red light. Phytochrome can absorbs red (660 nm) and far red (730 nm) light; involved in photomorphogenic and photoperiodic responses.
- seed germination in light requiring seeds. Some seeds will only germinate in the light, therefore sow on surface to see sunlight. Sunlight and any white or red light causes germination, but far red light inhibits germination.

6. 4.6. Photosynthetic rate and different water availability conditions

The environmental conditions have significant effects on the photosynthetic rates. Water is an essential factor in photosynthesis. Water effects the rate of photosynthesis because water is one of the reactants in the photosynthesis reaction. Slight deficiency of water results in significant reduction in the crop yield. The lack of water not only limits the amount of water but also the quantity of carbon dioxide. If there is not enough water pulled out of the ground via the roots and up to the plant through the xylem, the leaves and the whole plant might become dehydrated. If this happens, then the stomata on the leaves of the plant will close shut in order to conserve the water in the plant, as water is constantly exiting the plant through the stomata. When the stomata of the plant are shut, this also prevents CO2 in the air from entering the plant, and as a result, the rate of photosynthesis plummets. Lack of water can cause the plants to wilt, plants lose their ability to capture sunlight, so it limits photosynthesis at the biochemical level. In addition, if there is too much water in the soil, the roots will become rotten and die, causing the plant to die.

Under field condition severe water deprivation as drought stress can cause serious problems for plants. Drought stress can cause physiological, biochemical and molecular responses. The main physiological responses are recognition of root signal, loss of turgor and osmotic adjustment, reduced leaf water potential, reduced internal CO2 content, decline in net photosynthesis, reduced growth rates. Among biochemical responses, some severe responses can also be found: decrease in photochemical and RuBisCO efficiency, accumulation of stress metabolites like polyamines and proline, increase in activity of antioxidant enzymes. On molecular basis stress responsive gene expression can be found. Increased expression in abscisic acid biosynthetic gene can be revealed. The synthesis of specific proteins is increasing like late embryogenesis abundant proteins.

7. 4.7. References


4. Response of photosynthesis to light and different availability of water


www.sciencedirect.com


8. 4.8. Questions

1. Draw the light response curve of photosynthesis with its characteristic points!

2. Compare the light compensation point of C3 and C4 plants!

3. Compare the light compensation point of sun and shade plants!

4. List the non-photochemical dissipation process of photosynthesis!

5. Describe the xanthophyll cycle: localization, composition, operation under different light conditions!
5. Responses of photosynthesis to different soil nutrient supply and different leaf temperature conditions

1. 5.1. Photosynthesis – temperature curves

The response of photosynthesis to temperature is a central facet of plant response to climate change. Such responses have been found to be highly variable among species and among studies. When photosynthetic rate is plotted as a function of temperature in a leaf with C3 photosynthesis under ambient CO2 concentrations, the curve has a characteristic bell shape (Figure 11). The uphill part of the curve represents a temperature-dependent stimulation of enzymatic activities. The flat top portion of the curve represents a temperature range over which temperature is optimum for photosynthesis. The descent part is connected to temperature-sensitive deleterious effects, some of which are reversible while others are not.

5.1. ábra - Figure 11. Values of net-photosynthesis (%) as a function of temperature (Co)

The highest photosynthetic rates seen in temperature responses represent the so-called optimal temperature response. When these temperatures are exceeded, photosynthetic rates decrease again. It has been argued that this optimal temperature is the point at which the capacities of the various steps of photosynthesis are optimally balanced, with some of the steps becoming limiting as the temperature decreases or increases. The value of net-photosynthesis starts to decline beyond the temperature optimum. Respiration rates increase as a function of temperature, but they are not the primary reason for the sharp decrease in net photosynthesis at high temperatures. Rather, membrane-bound electron transport processes become unstable at high temperatures, cutting off the supply of reducing power and leading to a sharp overall decrease in photosynthesis.

In normal conditions, photorespiration increases with temperature in C3 plants, and the energy cost of net CO2 fixation increases accordingly. This higher energy cost is expressed in lower quantum yields at higher temperatures. Because of the CO2-concentrating mechanisms of C4 plants, photorespiration is low in these plants, and the quantum yield does not show temperature dependence. At lower temperatures, the quantum yield...
of C3 plants is higher than that of C4 plants, indicating that photosynthesis in C3 plants is more efficient at lower temperatures. C4 plants tend to have a higher photosynthetic temperature optimum than do leaves of C3 plants when grown under common conditions. Quantum yield as a function of temperature in a C3 plant and in a C4 plant. In the C4 plant the quantum yield or light-use efficiency remains constant with temperature, reflecting typical low rates of photorespiration. In the C3 plant the quantum yield decreases. With temperature, reflecting a stimulation of photorespiration by temperature and an ensuing higher energy demand per net CO2 fixed. While quantum yield effects are most expressed under light-limited conditions, a similar pattern is reflected in photorespiration rates under high light as a function of temperature. The combination of reduced quantum yield and increased photorespiration leads to expected differences in the photosynthetic capacities of C3 and C4 plants in habitats with different temperatures.

The responses to temperature are complex. Temperature affects all biochemical reactions of photosynthesis as well as membrane integrity in chloroplasts.

### 2. 5.2. Effect of high temperature

The real high temperature stress is defined as the rise in temperature beyond a critical threshold for a period sufficient to cause irreversible damage to plant growth and development.

The plasmalemma and membranes of cell organelles play a vital role in the functioning of cells. Any adverse effect of temperature stress on the membranes leads to disruption of cellular activity or death. Injury to membranes from a sudden heat stress event may result from either denaturation of the membrane proteins or from melting of membrane lipids, which leads to membrane rupture, and loss of cellular contents. Plant membranes exposed to high temperatures, total lipid content decreases to about one-half and the ratio of unsaturated to saturated fatty acids decreases to one-third of the levels at temperatures. Increase in saturated fatty acids of membranes increases their melting temperature and thus confers heat tolerance.

The effect of high temperature on higher plants is primarily on photosynthetic functions. The heat tolerance limit of leaves of higher plants coincides with the thermal sensitivity of primary photochemical reactions occurring in the thylakoid membrane system. Tolerance limits vary between genotypes, but are also subject to acclimation. Long-term acclimations can be superimposed upon fast adaptive adjustment of the thermal stability, occurring in the time range of a few hours. Light causes an increase in tolerance to heat, and this stabilization is related to the light-induced proton gradient. In addition to irreversible effects, high temperature may also cause large, reversible effects on the rate of photosynthesis.

Temperature effects on the rates of biochemical reactions by influencing enzyme activity. Failure of only one critical enzyme system can cause death of an organism. This fact may explain why most crop species survive sustained high temperatures up to a relatively narrow range, 40 to 45°C.

In addition to protecting the photosynthetic system against high light, the xanthophyll cycle may help protect against high temperatures. Chloroplasts are more tolerant of heat when they accumulate zeaxanthin. Thus, plants may employ more than one biochemical mechanism to guard against the deleterious effect of excess heat.

Heat stress may be an oxidative stress. Peroxidation of membrane lipids has been observed at high temperatures, which is a symptom of cellular injury. Enhanced synthesis of an anti-oxidant by plant tissues may increase cell tolerance to heat but no such anti-oxidant has been positively identified.

Synthesis and accumulation of proteins were ascertained during a rapid heat stress. These were designated as 'Heat Shock Proteins' (HSPs). Subsequently it was shown that increased production of these proteins also occurs when plants experience a gradual increase in temperature more typical of that experienced in a natural environment. Three classes of proteins as distinguished by molecular weight account for most HSPs, namely HSP90, HSP70, and low molecular weight proteins of 15 to 30 kDa (LMW HSP). The mechanism by which heat shock proteins contribute to heat tolerance is still not certain. One hypothesis is that HSP70 participates in ATP-dependent protein unfolding or assembly/disassembly reactions and those they prevent protein denaturation during stress. The LMW HSPs may play a structural role in maintaining cell membrane integrity during stress. Other heat shock proteins have been associated with particular organelles such as chloroplasts, ribosomes and mitochondria.

### 3. 5.3. Nutrient influence on photosynthesis
5. Responses of photosynthesis to different soil nutrient supply and different leaf temperature conditions

Photosynthesis is the main process in connection with plant production. Although photosynthesis works only with CO2 and water, a lot of nutrients are necessary to maintain plant ‘body’ and the background for several life events.

The availability of some plant nutrients is greatly affected by soil pH. The ideal soil pH is close to neutral, and neutral soils are considered to fall within a range from a slightly acidic pH of 6.5 to slightly alkaline pH of 7.5. It has been determined that most plant nutrients are optimally available to plants within this 6.5 to 7.5 pH range, plus this range of pH is generally very compatible to plant root growth.

Among nutrients nitrogen, phosphorous and potassium influence the rate of photosynthesis. Reduction in nitrogen supply adversely affects photosynthesis, as nitrogen forms the basic constituent of chlorophyll. Nitrogen is in every amino acid in a plant; thus, it must also be part of every single protein in a plant as well as being a major component of the chlorophyll molecule. Thus, nitrogen is involved in nearly every aspect of the light reactions as well as photosynthesis as a whole. Phosphorous also plays a big role in the light reactions of photosynthesis. It is phosphorous that is added to the ADP to form ATP which will be used elsewhere in the plant for energy. Phosphorous is also part of NADP, which is reduced to the NADPH2 that goes on to the Calvin cycle. Magnesium is the central component of the chlorophyll molecule and therefore is vital to the functioning of the light reactions of photosynthesis. Research has shown that up to ten percent of the magnesium in the plant is held in chlorophyll. Manganese, chlorine and possibly zinc are essential for the Hill reaction to function. Iron, sulfur and copper are all parts of proteins that help move electrons between the two photosystems.

4. 5.4. Connection between carbon and nitrogen cycle

Plants have a crucial role in connection between carbon and nitrogen cycle (Figure 12).

5.2. ábra - Figure 12. Connection between terrestrial carbon and nitrogen cycle (Thornton et al., 2009)

Plant carbon uptake by photosynthesis draws down atmospheric carbon dioxide (Atm CO2); litterfall and plant mortality pass biomass from plant to litter and coarse woody debris (CWD); decomposition of fresh litter generates soil organic matter; respiration by both plants and heterotrophic organisms returns CO2 to the atmosphere. Orange arrows show the additional processes represented in our coupled carbon-nitrogen land model, differentiated here between rapid internal cycling, and slower fluxes between land pools, the atmosphere, and ground water. The critical feedback pathway connecting heterotrophic respiration with plant growth is highlighted as a thick orange arrow: decomposition of soil organic matter not only releases CO2 to the atmosphere, it also releases nitrogen from the organic matter (mineralization) in forms that can then be taken up...
by plants (assimilation). Plant nitrogen uptake competes with the demand for mineral nitrogen from heterotrophic organisms decomposing fresh litter.

Within cells, integrating nutrient-specific pathways are vital to survival under constantly changing environmental and metabolic cues. It has been suggested that photosynthetic organisms accomplish this integration by tightly connecting photosynthetic processes to other principal metabolic pathways. Cellular carbon (C) and nitrogen (N) metabolism must be tightly coordinated to sustain optimal growth and development for plants and other cellular organisms (Figure 13). C and N metabolism are sinks for ATP and reducing power produced during photosynthesis. Protein complexes involved in the photosynthetic processes are in themselves a major metabolic sink for iron, S, N, and C. Similarly, intermediates of C and N metabolic pathways influence many other processes, including photosynthesis. Furthermore, photosynthetic processes capacitate several interconnected redox molecules that act as sensors for a number of metabolic pathways.

5.3. ábra - Figure 13. A simplified whole plant view of tightly coordinated C and N metabolism

C assimilation and N uptake occur in the leaf and the root systems, respectively. 2-oxoglutarate (2OG), an important intermediate product of C metabolism, serves as the C-skeleton for the synthesis of glutamate (which uses photorespiratory ammonium; not drawn here). Ammonium (NH4+) resulted from primary N assimilation from nitrate (NO3−) is then incorporated to glutamate, and glutamine is synthesized. Other amino acids are then synthesized by using NH4+ donated from glutamate and glutamine, and therefore proteins can be synthesized. Proteins are essential for almost all of cellular activities, including C and N metabolism. Despite the central role of maintaining an appropriate C/N balance or ratio in plants, the C/N balance sensing and signaling mechanisms remain largely unknown.

5. 5.5. References


www.global-warming-and-the-climate.com

5. Responses of photosynthesis to different soil nutrient supply and different leaf temperature conditions


Gregory, K. (2011): Climate Change Science, 2011. 08 September, Canada


6. 5.6. Questions

1. Draw the temperature response curve of photosynthesis with its characteristic points!

2. Compare the temperature response curve of photosynthesis in C3, C4 plants!

3. Find a relationship between the carbon and nitrogen cycles!
6. fejezet - 6. General and functional characteristics of the respiratory system

1. 6.1 Role and localization of respiration in a mitochondrion 'powerhouse'

Respiration is important because it provides metabolic energy as ATP and carbon skeletons for growth and maintenance for all living things. Heat production is important to some flowers because it helps to attract pollinators such as bees. Respiration is an essential component of a plant's carbon budget. During the process of respiration, the C-C bonds of complex compounds are breaking through oxidation within the cells, leading to release of considerable amount of energy. The compounds that are oxidised during this process are known as respiratory substrates. Usually carbohydrates are oxidised to release energy, but proteins, fats and even organic acids can be used as respiratory substances in some plants, under certain conditions. In all cases, the energy released by oxidation is used to synthesise ATP, which is broken down whenever (and wherever) energy needs to be utilised. ATP acts as the energy currency of the cells. In cellular respiration reaction 36 molecules of ATP are produced in complete oxidation of one molecule of glucose. During the process of respiration, oxygen is utilised, and carbon dioxide, water and energy are released as products. Respiration takes place in the cytoplasm and mitochondria in the cell of a living organism (Figure 14).

6.1. ábra - Figure 14. Schematic representation of the complex process of respiration in the mitochondrion

2. 6.2 Main reactions of plant respiration

The term glycolysis has originated from the Greek words, glycos for sugar, and lysis for splitting. In glycolysis, glucose is broken down with the help of enzymes and other molecules found in the cytoplasm. Enzymes first attach two phosphate groups to glucose to make it more reactive. The addition of the two phosphate groups prepares glucose for the action of another enzyme. This enzyme splits glucose in half to produce two three-carbon molecules, each with one phosphate group attached. In aerobic tissues, pyruvic acid produced during glycolysis is completely oxidized with the accompanying synthesis of much more ATP than in anaerobic glycolysis. The two ATP molecules gained in glycolysis are used for reactions in the cell that require energy.

Pyruvic acid oxidation takes place in the matrix of mitochondria by means of a cyclic sequence of reactions, the Krebs cycle/Citric acid cycle/Tricarboxylic Acid Cycle, which begins when the first product of pyruvate oxidation, acetyl coenzyme A, reacts with oxaloacetic acid to produce citric acid. The transition stage is a short biochemical pathway that ulinks glycolysis with the Krebs cycle. In this brief stage, enzymes transfer hydrogens and electrons from the two pyruvate molecules to two molecules of NAD+ to form two more molecules of NADH. Another enzyme breaks off one carbon and two oxygen atoms from each pyruvate molecule. These atoms combine to form carbon dioxide, the primary waste product of cellular respiration, which diffuses out of the cell. Because of these reactions, each pyruvate molecule is transformed into a two-carbon compound called...
an acetyl group. The two acetyl groups unite with two molecules of coenzyme A to form two acetyl coenzyme A molecules. The acetyl coenzyme A molecules are the molecules that enter the Krebs cycle. Oxaloacetic acid is eventually regenerated. Thus, the cycle can be repeated. During the Krebs cycle, the acetyl coenzyme A molecules are processed. As this complex pathway progresses, six molecules of NADH are formed. Additional carbon dioxide is created, and this process releases energy that is used to build two molecules of ATP from a pool of ADP and phosphate groups in the mitochondria. Hydrogens and electrons then are transferred to a molecule of flavin adenine dinucleotide (FAD++) to form FADH₂, a molecule like NADH that temporarily stores hydrogen and electrons for later use. By the end of the Krebs cycle, most of the usable energy from the original glucose molecule has been transferred to ten molecules of NADH (two from glycolysis, two from the transition stage, and six from the Krebs cycle); two molecules of FADH₂; and four molecules of ATP, two of which were formed in glycolysis.

The following steps in the respiratory process are to release and utilise the energy stored in NADH and FADH₂. This is accomplished when they are oxidised through the electron transport system and the electrons are passed on to O₂ resulting in the formation of H₂O. The metabolic pathway, through which the electron passes from one carrier to another, is called the electron transport system and it is present in the inner mitochondrial membrane. Electrons from NADH produced in the mitochondrial matrix during citric acid cycle are oxidised by an NADH dehydrogenase (complex I), and electrons are then transferred to ubiquinone located within the inner membrane. Ubiquinone also receives reducing equivalents via FADH₂ (complex II) that is generated during oxidation of succinate in the citric acid cycle. The reduced ubiquinone (ubiquinol) is then oxidised with the transfer of electrons to cytochrome-c via cytochrome-bc₁ complex (complex III). Cytochrome-c is a small protein attached to the outer surface of the inner membrane and acts as a mobile carrier for transfer of electrons between complex III and IV. Complex IV refers to cytochrome-c oxidase complex containing cytochromes-a and -a₃, and two copper centres. When the electrons pass from one carrier to another via complex I to IV in the electron transport chain, they are coupled to ATP synthase (complex V) for the production of ATP from ADP and inorganic phosphate. The number of ATP molecules synthesised depends on the nature of the electron donor. Oxidation of one molecule of NADH gives rise to 3 molecules of ATP, while that of one molecule of FADH₂ produces 2 molecules of ATP. Although the aerobic process of respiration takes place only in the presence of oxygen, the role of oxygen is limited to the terminal stage of the process. Yet, the presence of oxygen is vital, since it drives the whole process by removing hydrogen from the system. Oxygen acts as the final hydrogen acceptor. Unlike photophosphorylation where it is the light energy that is utilised for the production of proton gradient required for phosphorylation, in respiration it is the energy of oxidation-reduction utilised for the same process. It is for this reason that the process is called oxidative phosphorylation.

The pentose phosphate pathway meets the need of all organisms for a source of NADPH to use in reductive biosynthesis. This pathway consists of two phases: the oxidative phase, which generates NADPH and the nonoxidative interconversion of sugars. In the oxidative phase, NADPH is generated when glucose 6-phosphate is oxidized to ribose 5-phosphate. This five-carbon sugar and its derivatives are components of RNA and DNA as well as ATP, NADH, FAD and coenzyme A. The main purpose of the pentose phosphate pathway is to regenerate NADPH from NAPD⁺ through an oxidation/reduction reaction. This reaction is coupled to the formation of ribose 5-phosphate from glucose 6-phosphate. NADPH is used for reductive reactions in anabolism, especially in fatty acid synthesis. In red blood cells, the major role of NADPH is to reduce the disulfide form of glutathione to the sulphydryl form. The nonoxidative portion of the pathway creates carbon chain molecules ranging from 3 to 7 carbons. These compounds are intermediates in glycolysis and gluconeogenesis or other biosynthetic processes. The pentose phosphate pathway primarily produces NADPH, ribose 5-phosphate, fructose 6-phosphate, and glyceraldehyde 3-phosphate.

3. 6.3 Lipids as a substrates of respiration

Although glucose is the primary fuel for cellular respiration, cells can rely on other molecules to produce ATP. The cellular respiration pathway is connected to other metabolic pathways that can donate molecules to cellular respiration at different steps along the way. Fat are used as respiratory substrate after their hydrolysis to fatty acid and glycerol by lipase and their subsequent conversion to hexose sugar. Glycerol as a breakdown product of fat can enter the cellular respiration pathway in the middle of glycolysis. Lipids represent a more reduced form of carbon than carbohydrates, so the complete oxidation of 1 g of fat or oil can produce considerably more ATP (more, than two times) than the oxidation of 1 g of starch. In most seeds, triacylglycerols are stored in the cytoplasm of either cotyledon or endosperm cells in organelles known as oil bodies/spherosomes/oleosomes. The oil-body membrane is a single layer of phospholipids with the hydrophilic ends of the phospholipids exposed to the cytosol and the hydrophobic acyl hydrocarbon chains facing the triacylglycerol interior. The oil body is stabilized by the presence of specific proteins, called oleosins, that coat its outer surface and prevent the
phospholipids of adjacent oil bodies from coming in contact and fusing with it. In oilseeds, the conversion of lipids into sucrose is triggered by germination. It begins with the hydrolysis of triacylglycerols stored in oil bodies into free fatty acids, followed by oxidation of those fatty acids to produce acetyl-CoA.

The fatty acids are oxidized in a type of peroxisome called a glyoxysome. This organelle is also enclosed by a single membrane bilayer that is found in the oil-rich storage tissues of seeds. Acetyl-CoA is metabolized in the glyoxysome and cytoplasm to produce succinate, which is transported from the glyoxysome to the mitochondrion, where it is converted first into fumarate and then into malate. The process ends in the cytosol with the conversion of malate into glucose via gluconeogenesis, and then into sucrose. In most oilseeds, approximately 30% of the acetyl-CoA is used for energy production via respiration, and the rest is converted into sucrose. The initial step in the conversion of lipids into carbohydrates is the breakdown of triacylglycerols stored in oil bodies by the enzyme lipase, which hydrolyzes triacylglycerols into three fatty acid molecules and one molecule of glycerol. During the breakdown of lipids, oil bodies and glyoxysomes are generally in close physical association. The fatty acid molecules enter the glyoxysome, where they are activated by conversion into fatty-acyl-CoA by the enzyme fatty-acyl-CoA synthetase. The function of the glyoxylate cycle is to convert two molecules of acetyl-CoA into succinate (Figure 15).

6.2. ábra - Figure 15. Glyoxylate cycle and its connections

![Glyoxylate cycle diagram](image-url)

The acetyl-CoA produced by β-oxidation is further metabolized in the glyoxysome through a series of reactions that make up the glyoxylate cycle. Initially, the acetyl-CoA reacts with oxaloacetate to give citrate, which is then transferred to the cytoplasm for isomerization to isocitrate by aconitase. Isocitrate is reimported into the glyoxysome and converted into malate by two reactions that are unique to the glyoxylate cycle. First, isocitrate (C6) is cleaved by the enzyme isocitrate lyase to give succinate (C4) and glyoxylate (C2). The succinate is exported to the mitochondria. The second is, that malate synthase combines a second molecule of acetyl-CoA with glyoxylate to produce malate. Malate is then transferred to the cytoplasm and converted into oxaloacetate by the cytoplasmic isozyme of malate dehydrogenase. Oxaloacetate is reimported into the glyoxysome and combines with another acetyl-CoA to continue the cycle.

In cellular circumstances under which carbohydrates are scarce, plants can metabolize proteins as alternative respiratory substrates. Respiration of protein is less efficient than that of carbohydrate as assessed by the
6. General and functional characteristics of the respiratory system

respiratory quotient; however, under certain adverse conditions, it represents an important alternative energy source for the cell.

4. 6.4 Endogenous regulation of respiration

The rate of cell respiration is determined by supply and demand. If the cell is working hard, cell respiration speeds up, and when demand is not so high, respiration is slowed. Within the cell, there is an intricate network of enzymes that serve as signals to the cell to speed up or slow down (Figure 16). The most significant enzyme is phosphofructokinase, which spurs an early step in the respiration cycle. If ATP accumulates in the cell, phosphofructokinase is inhibited, slowing the process of respiration. As ATP is consumed, phosphofructokinase becomes active again and the respiration rate increases. This enzyme is also inhibited by an excess of citrate, which is produced during the Krebs cycle. The concentration of substrate also means a control over the respiratory processes.

6.3. ábra - Figure 16. The mechanism of ATP cycle

The ATP Cycle

5. 6.5. References


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6. 6.6. Questions

1. What are the main steps in aerobic respiration? Where does it take place?
6. General and functional characteristics of the respiratory system

2. Explain the mitochondrial electron transport system!

3. Compare the electron transport chains in mitochondria and chloroplasts with respect to membrane gradients and energy conservation!

4. Explain the role of pentose phosphate pathway!
7. fejezet - 7. Water in plant functioning

1. 7.1 Basic concepts: osmosis, diffusion, water potential

Water has several important properties, which makes this molecule extremely important for living organisms. Water is polar and rapidly forms hydrogen bonds. These two properties are the basis of most important role water plays. Water is a major component of cells and is a solvent for the uptake and transport of materials. It is a good medium for biochemical reactions, moreover water is a reactant in many biochemical reactions like photosynthesis. It can provide structural support via turgor pressure. Several plant movements are the result of water moving into and out of those parts such as diurnal movements, stomatal opening, flower opening. Water has a main role in cell elongation and growth and as thermal buffer.

To understand the behaviour and movement of water and its solvent in plant cell it needs to discuss three main concepts. First, the diffusion is the movement of molecules from an area in which they are highly concentrated to an area in which they are less concentrated. Second concept is the osmosis, which is a specialized case of diffusion, it represents the diffusion of a solvent (typically water) across a membrane. Water moves from a high concentration of water to a low concentration of water. This means that water would cross a selectively permeable membrane from a dilute solution to a concentrated solution. In biological systems, osmosis is essential since many biological membranes are semipermeable, and it leads to different physiological effects. The third concept is the water potential. Water potential is a measure of the energy state of water. This is a particularly important concept in plant physiology because it determines the direction and movement of water. Water potential (Ψ) is the chemical potential of water, compared to pure water at the same temperature and pressure. The units are in pressure because plant cells are under pressure and it is easier to measure pressure. Water potential is a complex of other potential: Ψ = Ψs + Ψp + Ψg + Ψm.

- **Solute potential** (Ψs): the effect of dissolved substances on the potential energy of a solution. It is defined as 0 MPa for distilled water.
- **Pressure potential** (Ψp): the effect of hydrostatic pressure on the potential energy of a solution
- **Gravitation potential** (Ψg): the effect of height of a system above sea level. It is defined as 0 MPa at sea level
- **Matric potential** (Ψm): the effect of colloids (adhesion) in soil or as a result of polymers in the cell wall

The water potential of pure water is zero. Water potentials in intact plant tissues are usually negative because of the large quantities of dissolved solutes in cells. The unit of water potential is measured in MPa. 1 MPa = 10 bars = 10 atm. Water always moves from the place with higher water potential to the place with lower water potential (Figure 17).

7.1. ábra - Figure 17. Gradient of water potential in plants
2. 7.2 Transport of water

2.1. 7.2.1. Water movement between cells

Each cell has pores, the so called plasmodesmata, in its membrane where the plasma can make a connection between the adjacent cells. The cytoplasm of all the cells in the plant connected by the plasmodesmata is called the symplast; if particles travel through the symplast they gain access to the cell’s interior and the entire plant body. This transport way is called symplastic pathway. In contrast to the symplast, some particles are not allowed into the inner membrane pathway and instead move through the apoplast, which is the cell walls and the intracellular spaces within the plant. This way is called apoplastic pathway. These two pathways regulate the substances that are allowed to travel through the plant.

2.2. 7.2.2. Water transport from soil to leaf

The entry of water into the root hair dilutes the cell sap. Soil water enters the root through its epidermis. It appears that water then travels in both apoplastic and symplastic pathways.

7.2. ábra - Figure 18. The way of water from soil to xylem in the plant root

The inner boundary of the cortex, the endodermis, is impervious to water because of a band of lignified matrix called the Casparian strip (because of suberin). Therefore, to enter the stele, apoplastic water must enter the symplast of the endodermal cells. From here it can pass by plasmodesmata using symplastic way into the cells.
of the stele. Once inside the stele, water is again free to move between cells as well as through them. In young roots, water enters directly into the xylem vessels and/or tracheids. In the xylem, water with the minerals that have been deposited in it (as well as occasional organic molecules supplied by the root tissue) move up in the vessels and tracheids. At any level, the water can leave the xylem and pass laterally to supply the needs of other tissues. At the leaves, the xylem passes into the petiole and then into the veins of the leaf. Water leaves the finest veins and enters the cells of the spongy and palisade layer. Here some of the water may be used in metabolism, but most of it is lost in transpiration through stomata.

3. 7.3 Permanent osmoregulation, root pressure

Root pressure, in plants helps to drive fluids upward into the water-conducting vessels. It is primarily generated by osmotic pressure in the cells of the roots and can be demonstrated by exudation of fluid when the stem is cut off just aboveground. The water uptake is indirectly active, because plant cell takes up ions first – permanent osmoregulation – to make water potential differences between root cell and soil solution. Ion uptake is an active process, which uses ATP produced by mitochondrial electron transport. It is partially responsible for the rise of water in plants. Root pressure causes guttation, the exudation of water droplets that can be seen in the morning on the tips of grass blades or the leaf margins of some small, herbaceous dicots. Root pressure can force water upward only few metres. Transpiration provides the pull, and the cohesion of water due to hydrogen bonding transmits the upward pull along the entire length of the xylem to the roots. The mechanism of transpiration depends on the generation of negative pressure in the leaf due to unique physical properties of water.

4. 7.4. Transpiration

Transpiration is the process by which moisture is carried through plants from roots to small pores on the underside of leaves, where it changes to vapor and is released to the atmosphere. Transpiration is essentially evaporation of water from plant leaves. Transpiration also includes a process called guttation, which is the loss of water in liquid form from the uninjured leaf or stem of the plant, principally through water stomata (hydratodes). The roles of transpiration are: supplying photosynthesis (1%-2% of the total), bringing minerals from the roots for biosynthesis within the leaf and cooling the leaf.

There are a number of factors that determine transpiration rates. Plants transpire more rapidly in the light than in the dark. This is largely because light stimulates the opening of the stomata. Light also speeds up transpiration by warming the leaf. Transpiration rates go up as the temperature goes up, especially during the growing season, when the air is warmer due to stronger sunlight and warmer air masses. Higher temperatures stimulate the stomatal opening and water is released to the air, whereas colder temperatures cause the stomatal pores to close. As the relative humidity of the air surrounding the plant rises the transpiration rate falls. It is easier for water to evaporate into dryer air than into more saturated air, because of differences of water potential. Increased movement of the air around a plant will result in a higher transpiration rate. This is somewhat related to the relative humidity of the air, in that as water transpires from a leaf, the water saturates the air surrounding the leaf. If there is no wind, the air around the leaf may not move very much, raising the humidity of the air around the leaf. Wind will move the air around, with the result that the more saturated air close to the leaf is replaced by drier air. When there is a lack of moisture, plants can begin to senesce and transpire less water. Different plant species transpire water at different rates. Some plants, which grow in arid regions, such as cacti and succulents, conserve precious water by transpiring less water than other plants.

5. 7.5 Stoma function

The exchange of oxygen, carbon dioxide and the loss of water in the leaf occur through pores called stomata. Guard cells are cells surrounding each stoma. Guard cells drive stomatal movement through changes in their osmotic pressure. When the guard cells are turgid the stomatal pore is large. This turgidity is caused by the accumulation of potassium ions in the guard cells. As potassium ion levels increase in the guard cells, the water potential of the guard cells drops, and water enters the guard cells. This actively accumulates potassium ions from neighboring epidermal cells due to proton pump-generated membrane potential. Part of this response is due to the activation of blue-light dependent receptors that transiently activate the plasma membrane H+-ATPase.

When the guard cells have lost water, it causes the cells to become flaccid and the stomatal pore to close. This may occur when the plant has lost an excessive amount of water. In addition, it generally occurs daily as light levels drop and the use of CO2 in photosynthesis decreases.
Stomatal opening is tightly controlled to meet the demand for CO2 for photosynthesis and restrict the loss of water during drought. On hot and sunny days, guard cells will often receive conflicting signals, high light promoting stomatal opening and low humidity inducing stomatal closure. This raises the question, how guard cells process these signals in such a way that opposing responses are prevented. Guard cell responses to blue light and ABA are clearly time dependent. After receiving these signals, guard cells show a maximal response after a few minutes, which levels off afterwards. Once the response has passed its maximum guard cells transport can be altered again by other signals. The response to photosynthetic light differs in this respect, since here a negative feedback system couples the influx of CO2 through stomata to the demand for CO2 in the leaf.

There are many signals that induce stomatal closure, among these the best known signal is probably ABA. In the signaling pathway towards stomatal closure, there are several secondary messengers, such as Ca2+, H2O2 and NO that contribute to the stomatal closure. Passive loss of turgor pressure also results in stomatal closure. Hydro passive stomatal closure occurs when the water evaporation from the guard cells is too low to be balanced by water movement into these cells. The water content in the cells is then rapidly reduced to the extent where the osmotic pressure is reduced and the cells lose turgor pressure and shrink. When this happens the guard cells are unable to maintain the shape and the stomatal pore is covered. Passive stomatal closure is important in ferns and Lycopods.

6. 7.6. References


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7. 7.7. Questions

1. What is the importance of the water potential concept in plant physiology?

2. Explain the method of horizontal water transport!

3. Describe permanent osmoregulation in the root!

4. Analyse the role of transpiration!
8. fejezet - 8. Acquisition of nutrients, membrane and long way transport

The beneficial effect of adding mineral elements (e.g., plant ash or lime) to soils to improve plant growth has been known in agriculture for more than 2,000 years. Nevertheless, even 150 years ago it was still a matter of scientific controversy as to whether mineral elements function as nutrients for plant growth. It was mainly to the credit of Justus von Liebig (1803–1873) that the scattered information concerning the importance of certain elements for plant growth was compiled and summarized and that the mineral nutrition of plants was established as a scientific discipline. These achievements led to a rapid increase in the use of mineral fertilizers. By the end of the nineteenth century, especially in Europe, large amounts of potash, superphosphate and, later, inorganic N were used in agriculture and horticulture to improve crop growth and production.

Liebig’s conclusion that the elements N, S, P, K, Ca, Mg, Si, Na and Fe are essential for plant growth was reached by observation and speculation rather than by precise experimentation. The fact that the ‘mineral element theory’ was based on this unsound foundation was one of the reasons for the large number of studies undertaken at the end of the nineteenth century. From these and other extensive investigations on the elemental composition of different plant species growing on various soils, it was realized as early as the beginning of the last century that neither the presence nor the concentration of an element in a plant is a criterion for essentiality. Plants have a limited capability for the selective uptake of those elements, which are essential for their growth. Additionally they take up elements which are not needed for growth and which may even be toxic.

The term essential mineral element (or mineral nutrient) was proposed by Arnon and Stout (1939). These authors concluded that, for an element to be considered essential, three criteria must be met:

1. A given plant must be unable to complete its lifecycle in the absence of the element
2. The function of the element must not be replaceable by another element.
3. The element must be directly involved in plant metabolism – for example, as a component of an essential plant constituent such as an enzyme – or it must be required for a distinct metabolic step such as an enzyme reaction.

According to this strict definition, an element which alleviates the toxic effects of another element (e.g., Si for Mn toxicity), or one which simply replaces another element (e.g., Na for K) may not be described as essential for plant growth.

1. 8.1 Composition of biological membranes

The capacity of plant cell membranes to regulate solute uptake has fascinated botanists since the nineteenth century. By the early years of the twentieth century some basic facts of solute permeation across biological membranes had been established, such as the inverse relationship between the diameter of uncharged molecules and the rates at which they permeate membranes. High-molecular-weight organic solutes such as polyethylene glycol are not taken up by cells and can be used at high external concentrations as osmotica to induce water deficiency (drought stress) in plants. However, some hydrophobic molecules penetrate membranes much faster than would be predicted based on their size, which is presumably related to their ability to partition into the lipid bilayer.

Biological membranes are typically composed of a lipid bilayer and associated proteins (Fig. 19).

8.1. ábra - Figure 19. Structure of biological membrane
However, membrane composition is sensitive to environmental conditions, and the relative abundance, and types, of both lipids and proteins in membranes surrounding cellular compartments differ. The lipids in cell membranes have hydrophilic headgroups and hydrophobic tails. The most abundant membrane lipids are:

- phospholipids, in which the hydrophilic headgroup is  \textit{linked} to the hydrophobic tail by a phosphate group,
- sterols, which are based around a four-ring structure, and
- glycolipids, which have sugars as their hydrophilic headgroup.

Common plant phospholipids are phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl inositol and diphosphatidyl glycerol. Plasma membranes and mitochondria are enriched in phosphatidyl inositol and diphosphatidyl glycerol, respectively. The fatty acid moiety in phospholipids varies in both chain length and number of double bonds, but is often palmitic (length:double-bonds, 16:0), stearic (18:0), oleic (18:1), linoleic (18:2) or linolenic (18:3) acid. Major plant sterols include campesterol, sitosterol and stigmasterol. The sterol content of the ER is low, but sterols can make up more than 30% of the total lipids in the plasma membrane and tonoplast. Most glycolipids are found in the chloroplast, where the thylakoid membrane is predominantly composed of monogalatosyldiacylglycerol (MGDG), together with digalatosyldiacylglycerol (DGDG) and the sulpho-lipid, sulphoquinovosyldiacylglycerol (SQDG).

Although the lipid bilayer provides the basic structure of the membrane and forms a permeability barrier, most biological functions of membranes are performed by proteins. The membrane surrounding each cellular compartment has different types of proteins reflecting the particular function of that membrane. Membrane proteins function (i) to anchor the membrane to the cytoskeleton and/or cell wall, (ii) as receptors/transducers for compartmentalized signals, (iii) as enzymes for specific reactions, such as energy transduction processes in mitochondria and chloroplasts, and (iv) to transport specific solutes across membranes.

There are several ways by which proteins can be associated with the lipid bilayer. Many membrane proteins extend through the bilayer. These integral transmembrane proteins have both hydrophobic and hydrophilic portions. Their hydrophobic portions lie within the bilayer, alongside the hydrophobic tails of the lipid molecules, while their hydrophilic portions extend into the aqueous environment on either side of the membrane. Other membrane proteins are located entirely outside the bilayer. These peripheral proteins are
bound to the membrane through lipid groups attached covalently through prenylation (attachment of the isoprenoids farnesylphosphate or geranylgeranylphosphate), S-acetylation (attachment of palmitate or stearate) or N-myristoylation, or are associated with other membrane proteins through ionic interactions. It is thought that lipid modification of membrane proteins also facilitates their subcellular targeting and clustering into specific domains.

Lipid composition not only differs between cellular membranes, plant tissues and plant species, but also is strongly influenced by environmental factors. In leaves, for example, distinct annual variations in sterol concentrations occur, membrane lipid composition changes during exposure to low temperatures and DGDG and SQDG can replace phospholipids in membranes of P deficient plants. Similarly, the composition of root membranes is influenced by temperature, salinity and the ionic composition of the external solution. The changes in lipid composition reflect often the adaption of a plant to its environment. For example, membranes of plants growing at low temperatures have more phospholipids with charged headgroups and shorter fatty acid chains with lower degree of saturation, and greater sterol content than plants growing at higher temperatures. Such changes shift the freezing point (i.e., the transition temperature) of membranes to a lower temperature and may therefore be important for the maintenance of membrane functions at low temperatures.

Cellular membranes are dynamic structures that are continuously remodelled to allow the plant to respond to developmental signals, biotic challenges, and environmental conditions. This remodelling occurs over minutes to months, and is supported by complex trafficking pathways that deliver lipids and proteins to and from cellular membranes. These pathways are functionally unlinked through the Golgi apparatus to the endoplasmic reticulum, plasma membrane, peroxisomes, vacuoles, mitochondria and chloroplasts. The delivery of secretory vesicles to the plasma membrane can be targeted to specific locations, such as the apex of tip-growing cells, e.g. elongating root hairs or pollen tubes, or to plasmodesmata. Thus, membranes are not homogeneous, but possess domains in which specific lipids and proteins can be clustered, stably or transiently, to improve the efficiency of biochemical and physiological processes.

2. **8.2 Solute transport across membranes**

K concentration in maize root sap (which is approximately equal to the K concentration of the vacuoles) was 80 times higher than in the external solution. In contrast, the Na concentration in the root sap remained lower than that in the external solution. Such phenomena require both a source of energy and selective transport across the plasma membrane of root cells.

Transport across plant membranes is facilitated by transmembrane proteins. These can be classified into three groups: (i) primary active transporters (pumps), in which solute transport is coupled directly to the hydrolysis of an energy substrate such as ATP or pyrophosphate (PPI); (ii) secondary active transporters or ‘coupled transporters’, which harness the electrochemical gradient of (generally) H+ to the movement of a solute in either the same (symport) or opposite (antiport) direction; and (iii) passive transporters, which catalyse the movement of solutes down their electrochemical gradient. The latter group includes a variety of carriers (uniporters) and channels. Channels can be distinguished from uniport carriers by their high catalytic rate, which can exceed 10 million ions s⁻¹ which is several orders of magnitude greater than uniport carriers. In the next paragraphs, the driving forces for solute movement across membranes are considered in relation to facilitated diffusion, or ‘passive’ transport, of solutes down their electrochemical gradient by carriers and channels, and to ‘active’ transport of solutes against their electrochemical gradient catalysed by pumps and coupled transporters.

Under most circumstances, the driving force for the facilitated diffusion of an uncharged solute across a membrane is its concentration gradient, whereas for an ion it is its electrochemical gradient. The Nernst equation allows the direction of the net diffusive flux of an ion at a given membrane potential and temperature to be determined. When the cell membrane potential is more negative than the Nernst potential, captions can move into the cell, and anions out of the cell, by facilitated diffusion. When the membrane potential is more positive than the Nernst potential, the opposite fluxes are favoured. According to the Nernst equation, at 20°C with a membrane potential of 100 mV, K⁺ or Cl⁻ would be in electrochemical equilibrium across the plasma membrane if their concentration in the cytosol were 52 times higher (K⁺) or 52 times lower (Cl⁻) than in the external solution. At the same temperature and membrane potential, the concentrations of a divalent cation or anion would differ more than 2,700-fold between the cytosol and external medium if it was in electrochemical equilibrium.

The resting membrane potential of root cells is often more negative than 2100 mV. It is generated primarily by the activity of plasma membrane H⁺-ATPases encoded by members of the AHA gene family. These H⁺ pumps...
are clustered in discrete (micro)domains of the plasma membrane and their activity is regulated by phosphorylation-dependent interactions with cytosolic 14-3-3 proteins in response to diverse environmental signals including exposure to salt and low temperatures. Under physiological conditions, many cations are in electrochemical equilibrium across the plasma membrane of root cells. However, there is always a large electrochemical gradient driving Ca2+ influx to cells, and, in saline environments, there is also a large electrochemical gradient driving Na+ influx. On the other hand, anions cannot be concentrated in the cytoplasm by facilitated diffusion across the plasma membrane, and their influx to root cells is often facilitated by symporters coupled to the proton electrochemical gradient generated by plasma membrane H+-ATPases.

At the molecular level, facilitated diffusion is mediated by uniporters or channels. Passive transporters facilitating the influx of 10 of the 14 mineral nutrients across the plasma membrane of root cells have been reported (Fig. 20.). These include K-channels, voltage-dependent Ca-channels and cation channels.

A multitude of secondary active transporters are present in the plasma membranes of root cells, which couple H+ influx to the movement of solutes against their electrochemical gradients. Proton-coupled transporters in the plasma membrane of root cells are responsible for the uptake of anions, such as nitrate (e.g., NRT1 and NRT2 transporters), phosphate (e.g., PHT1 transporters), sulphate (SULTR1 transporters), chloride and (probably) molybdate. Proton-coupled transporters also alleviate element toxicities by removing chloride, sodium and boron from root cells. In the stele, proton-coupled transporters load nitrate and B into the xylem. Similar transport proteins are present in the plasma membranes of other plant cells, where they serve both general and specific functions. The transport of amino acids, peptides and sugars across the plasma membrane is also catalysed by proton-coupled transporters.

The tonoplast of the vacuole similarly contains a variety of primary active transporters, proton-coupled transporters, uniporters and channels (Fig. 20).

8.2. ábra - Figure 20. The possible ways of transmembrane transport (uniport, symport, antiport)

In cells of higher plants, the electrical potential difference between the vacuole and the cytosol is about -20 to -60 mV and the pH of the vacuolar sap can be as low as pH 3. Based on estimates of solute concentrations in the cytosol and vacuole, it is thought that sequestration of K+, Na+, Ca2+, Mg2+, Zn2+, Mn2+ and nitrate requires active transport into the vacuole, whereas the movement of other anions is likely to be passive.

The tonoplast contains two distinct types of proton pumps, the H+-ATPases and the H+-PPiases that generate the negative electrical potential across the tonoplast and lower the pH of the vacuole.

The tonoplast also contains Ca2+-ATPases (e.g., AtACA4) that pump Ca2+ into the vacuole and a variety of ATP Binding Cassette (ABC) transporters that protect the cytoplasm by removing heavy metals, oxidation products conjugated to glutathione and xenobiotics from the cytosol into the vacuole. These transporters are also involved in the sequestration of chlorophyll catabolites and natural pigments in the vacuole.

Several ion channels have been recorded in the tonoplast. These facilitate the movement of K+, Cl-, NO3-, ammonia, amino acids, urea, Ca2+, SO42-, HPO42-, sugars and organic acids in the direction of their electrochemical gradients. The rapid efflux of K+ and Cl- from the vacuole, through fast vacuolar (FV), slow vacuolar (SV) or vacuolar potassium (VK) channels and Cl- channels, respectively, is required for stomatal closure and other osmotically driven plant movements. The sequestration and release of NO3-, ammonia, amino acids and urea are central to the N economy of plants. Aquaporins have been shown to facilitate the transport of ammonia and urea across the tonoplast.
From the preceding discussion, it is apparent that proton pumps are responsible for energizing solute transport across cell membranes. However, it is important to note that these pumps not only generate the proton electrochemical gradient across the tonoplast and plasma membrane, and the acidic conditions of the apoplasm (pH ~5.5) and the vacuole (pH 4.5–5.9), but also maintain cytosolic pH at its optimal value (pH 7.3–7.6).

3. 8.3 Uptake of ions and water along the root axis

Roots vary both anatomically and physiologically along their longitudinal axes. This should be borne in mind when models for ‘the’ behaviour of root tissue and root cells are based on studies with isolated roots or roots of intact plants. In the apical zone, non-vacuolated cells dominate. These cells differ in many respects from the vacuolated cells in the basal zones. The apical root zones have higher respiration rates, which decrease rapidly when the carbohydrate supply to roots is interrupted, for example following excision. In general, there is a tendency for the rate of ion uptake per unit root length to decrease with distance from the root apex. However, this tendency strongly depends on the identity of the ion, plant nutritional status and plant species. When K or Ca are supplied to different regions of seminal roots of maize, the uptake rate of K is slightly lower in the apical zone than the sub-apical zone, despite the high K requirement for growth. The high K concentration in root apical cells of about 200 mM is maintained not only by uptake from the external solution but also by delivery from more basal root zones or from the shoot via the phloem. Similar observations have been made in other cereals and also in non-mycorrhizal long roots of perennial plant species such as Norway spruce.

In contrast to K the uptake of Mg, and particularly of Ca, is higher in apical than in basal root zones. Because Ca mobility in the phloem is low, apical cells of the root must meet their Ca demand for growth by direct uptake from the external solution. Root apical zones also contribute considerably to Ca delivery to the shoot. At the root tip, Ca may reach the xylem through an exclusively apoplastic pathway or may be transported across the Casparian band through immature, unsuberized endodermal cells. Calcium delivery to the xylem is also high in basal root zones, where lateral roots emerge from the pericycle, disrupting the integrity of the Casparian band. The apoplastic pathway is also important for the movement of Na, Zn, Fe and Cd to the xylem. The delivery of these elements to the xylem is often greatest at the root tip.

The decline in P uptake along the root axis is much less striking than that for Ca. In soil-grown maize this decline is mainly related to a decrease in root hair viability and, thus, in absorbing root surface area. The gradient in P uptake along the root axis also depends on the P nutritional status of the plant and may be reversed under deficiency in favour of the basal zones. The situation is different under Fe deficiency in Strategy I plants where the apical, but not the basal, root zones increase their capacity for Fe uptake by a factor of up to 100. Apical, or immediately sub-apical, root zones generally contribute most to nitrate and ammonium uptake by intact plants irrespective of their nutritional status, although the magnitude of the decline in uptake with distance from the root apex depends greatly on root anatomy. Indeed, it should be noted that the uptake of most elements is restricted when the rhizodermis and cortex cells of basal (older) regions of the roots collapse and die.

Formation of cortical gas spaces (aerenchyma) particularly in more basal root zones can often be observed. The formation of aerenchyma is a typical response to oxygen deficiency in the root zone in plant species adapted to wetland conditions, but it can also be induced, for example, in maize roots under fully aerobic conditions by temporary deprivation of N or P supply. Despite these anatomical changes, the basal root zones still have a considerable capacity for ion uptake and for radial transport, indicating that the strands of cells bridging the cortex maintain sufficient ion transport capacity from the rhizodermis to the endodermis.

8.3. ábra - Figure 21. Segment of a transverse section of a maize root showing (A) symplasmic and (B) apoplastic pathways of solute movement across the root
Water uptake can affect ion uptake both directly, through effects on the rate of radial transport of ions through the apoplasm, and indirectly, by influencing the supply of ions to the plasma membrane of root cells. Water uptake is usually low at the extreme root apex, but increases in the elongation zone and reaches a maximum in the root hair zone, where the endodermis is undergoing suberization. Water uptake is often reduced strongly following suberization of the endodermis and, particularly, the exodermis. Water can reach the xylem through both the apoplast and via root cells. Transport through root cells is facilitated by aquaporins. Aquaporins are found in various membranes of root cells, including the plasma membrane and the tonoplast. Recent data, using mercury to inhibit the activity of aquaporins, suggest that rapid changes in root hydraulic conductivity in response to many stimuli, such as diurnal cycles, nutrient deficiency, salt stress, low temperatures, anoxia and drought, are the result of changes in cell membrane permeability achieved by regulation of aquaporin activity. The abundance of aquaporins is often greatest in the elongation and mature root zones. In these root zones, strong expression of genes encoding aquaporins is observed in the endodermis and exodermis, presumably to allow water to bypass the Casparian band through a transcellular pathway.

4. 8.4 Radial transport of ions and water across the root

There are two parallel pathways of movement of solutes and water across the cortex towards the stele: one passing through the apoplasm (cell walls and intercellular spaces) and another passing from cell to cell in the symplasm through the plasmodesmata (Fig.22.).

8.4. ábra - Figure 22. The apoplast and symplast way in plant

In most of the root, the apoplastic movement to the stele is restricted by the Casparian band in the walls of endodermal cells. This band is suberized and joins each endodermal cell (stage I endodermis). In the basal regions of the root, suberin lamellae cover the entire surface of endodermal cells (stage II endodermis). This prevents endodermal cells taking up solutes from the apoplasm. Thick cellulose secondary walls are deposited over the suberin lamellae, which can be lignified (stage III endodermis). The nature and extent of these cell wall modifications are determined by both genetic and environmental factors.
In most angiosperms, another apoplasmic barrier, the exodermis, can develop in parallel with the endodermis. The exodermis develops in the same three stages as the endodermis. Formation of an exodermis is found, for example, in Zea mays, Allium cepa, or Helianthus annuus, but not in Vicia faba or Pisum sativum. However, there are somewhat different views on the function of the exodermis as an effective barrier for transport of water and solutes in the apoplasm of the root cortex. Termination of the apoplasmic pathway at the exodermis, would confine the entry of solutes and water to the root symplast to the rhizodermal cells in basal root zones. Although rhizodermal cells, and in particular root hair cells, play a key role in the acquisition of mineral nutrients, especially K and P, the relative importance of the two pathways for solute transport across the root cortex is unknown. It will depend on: (i) the external concentration versus the capacity and affinity of the transport system for a particular solute at the plasma membrane of root cells; (ii) the root zone considered: depending on environmental conditions and the growth rate of the root, the exodermis can develop within a centimetre of the root apex or remain undeveloped and may possess ‘passage cells’; and (iii) the hydraulic conductivity of the root zone considered and the transpiration rate of the shoot. For water, estimates of the contribution of the apoplasmic pathway to radial transport across roots vary between about 10 and 70%.

The endodermis is also not a perfect barrier to the apoplasmic movement of water and solutes from the cortex to the stele. In addition to the presence of passage cells in some plant species, this barrier may be ‘leaky’ at two sites along the root axis, at least. At the root apex, where the Casprian band is not yet fully developed, the apoplasmic movement of water and solutes to the stele can occur. However, the movement of some solutes, such as polyvalent cations like aluminium, through the apoplasm of the root apex can be restricted by mucilage formed at the external surface of the rhizodermal cells. The apoplasmic pathway to the stele is also possible in basal root zones where the structural continuity of the endodermis is disrupted transiently by the emergence of lateral roots from the pericycle, as has been demonstrated, for example, for Ca, Al and water. This ‘bypass-flow’ becomes particularly important for water supply to the shoot at high transpirational demand and in the accumulation of Na in leaves under saline conditions. Both genetic and environmental factors influence the movement of water and solutes via the apoplasmic pathway through their effects on the development of the endodermis and exodermis. Accelerated deposition of suberin and lignin restricts the apoplasmic movement of cations and other solutes to the xylem and reduces hydraulic conductivity.

The symplasmic pathway plays a key role in delivering most nutrients to the xylem, beginning either at the rhizodermis and the root hairs, at the exodermis, or at the endodermis. Radial transport in the symplasm requires movement through plasmodesmata, which connect neighbouring root cells. Plasmodesmata have a complex structure. The simplest type, which occurs in young tissues, comprises a tube of appressed endoplasmic reticulum (ER) running through the pore, the desmotubule. The transport of solutes and water between cells occurs in the ‘cytoplasmic sleeve’, i.e. the cytosol between the desmotubule and the plasma membrane. Protein structures in the cytoplasmic sleeve create microchannels through which solutes can diffuse. In more mature tissues, the structure becomes more complex through the addition of branches and the formation of central cavities. Plasmodesmata can be closed and opened by the production and degradation of a ‘collar’ of callose (β-1,3-glucan) and they generally have a size exclusion limit of about 1 kDa, which is regulated physically by the collar and also by interactions with cytosolic proteins. Indeed, plasmodesmal microchannels can dilate to allow the passage of solutes in excess of 20 kDa. The primary role of plasmodesmata appears to be cell communication, as they regulate the transport of transcription factors and microRNAs that control plant development and responses to biotic and environmental challenges. Additionally, the regulation of plasmodesmal conductance represents another mechanism of cellular control of ion fluxes across the root.

High cytosolic Ca2+ concentrations induce closure of plasmodesmata and many environmental stimuli that increase cytosolic Ca2+ also disrupt the symplasmic movement of water and nutrients across the root. The number of plasmodesmata per cell varies considerably between plant species and cell type. Rhizodermal cells that have developed into root hairs generally have more plasmodesmata than other rhizodermal cells. The relatively small number of plasmodesmata in Raphanus raises the question as to whether the root hairs are of major importance for symplasmic radial transport in this plant species. However, not only the number of plasmodesmata, but also whether they are functional must be taken into account. In the endodermis of young barley roots, on average 20,000 plasmodesmata per cell have been found. In the tertiary (lignified) endodermis of older zones of barley roots, there are far fewer plasmodesmata, but the number appears to be sufficient to permit considerable radial transport of both water and ions through the endodermis.

The mechanism of symplasmic transport of solutes seems to be chiefly by diffusion, facilitated by radial water flux and cytoplasmic streaming. During their radial transport through the symplasm, elements can be metabolized and/or sequestered in the vacuoles of root cells. When a nutrient is supplied to roots of a plant that is deficient in that nutrient (‘low-salt’ roots), it is accumulated in vacuoles of root cells resulting in an immediate accumulation in roots and a delay in its translocation from the roots to the shoots. Thus, when the supply of a
nutrient is suboptimal, the roots usually have higher tissue concentrations of that particular nutrient than the shoot. In long-term studies, this phenomenon is responsible, in part, for the often observed shift in the relative growth rates of roots and shoots in favour of the roots under nutrient deficiency.

The vacuoles of root cells also remove potentially toxic elements from the symplasmic pathway. For example, vacuolar sequestration of Na\(^+\) in the root accounts for the restricted shoot transport of Na\(^+\) in natrophobic plant species. Preferential accumulation in roots also restricts the translocation of Ca, Mo, Cd and Al to the shoot. In contrast, in plants sufficiently supplied with P, symplasmic transport of P, and its translocation to the shoot, is greater than accumulation in root vacuoles. The exchange rate between ions in the vacuoles of cortex cells and those in the symplasm depends on the ion species (K\(^+\) > Na\(^+\); NO\(_3^-\) > SO\(_4^{2-}\)), and the half-time for exchange is generally in the order of at least a few days.

8.5. ábra - Figure 23. The radial transport of water and nutrients

The radial transport of water and solutes is strongly influenced by maturation of the xylem vessels along the root axis (Fig. 23.). For example, in graminaceous species such as maize growing in soil, two root zones can be observed: ‘sheathed’ zones, which are covered by a layer of strongly adhering soil, the rhizosheath and ‘bare’ zones. The development of the rhizosheath appears to be related to the presence of root hairs. In the sheathed zones, the metaxylem vessels are still alive and non-conducting, whereas in the bare zones the metaxylem is mature. Accordingly, the hydraulic conductivity of bare roots is about 100 times greater than that of sheathed roots. This difference in hydraulic conductivity and thus water uptake results in high water contents in the rhizosphere soil of the sheathed zones and low water contents in the rhizosphere soil of the bare zones. Living metaxylem vessels can be found up to 20–30 cm proximal to the root tip in maize, and up to 17 cm proximal to the root tip in soybean. This delay in metaxylem maturation not only affects hydraulic conductivity of the roots and plant water relations but also the movement of solutes to the xylem and their translocation to the shoot.

5. 8.5 Release of ions into the xylem

After radial transport through the symplasm to the stele, ions and organic solutes (amino acids, organic acids) are released into the xylem. This release (xylem loading) into fully differentiated, non-living xylem vessels occurs across the plasma membrane of xylem parenchyma cells. The membrane potential of these cells is slightly negative and the xylem sap has a pH between about 5.2 and 6.0. Solute enter the xylem through ion channels or uniporters, if their electrochemical gradients allow this, or their transport is coupled to the proton electrochemical gradient generated by the plasma membrane H\(^+\)-ATPase or directly to ATP hydrolysis. The
xylem parenchyma cells are also responsible for the reabsorption of solutes from the xylem sap by tissues along the pathway to the shoot.

The key role of the H+-ATPases in the plasma membrane of parenchyma cells in xylem loading is now well established. Protons are pumped into the xylem both to generate a negative membrane potential and to acidify the xylem sap. The K+ electrochemical gradient is sufficient for K to be loaded into the xylem by voltage-gated, outwardly rectified K-channels. Similarly, anion channels can facilitate the movement of nitrate, sulphate, phosphate and chloride from the symplasm to the xylem in the direction of their electrochemical gradients. In addition, nitrate can be loaded into the xylem by members of the NRT1 (nitrate transporter 1) family. Cations present at low concentrations in the root symplasm are loaded into the xylem by active transport mechanisms. It is thought that Mg and Mn are also loaded into the xylem by ATPases, although the genes encoding these transporters are not yet known. The regulation of xylem loading separately from solute uptake offers additional possibilities to control the selectivity and rate of long-distance transport to the shoot, for example in response to shoot demand.

Separate genetic control of solute uptake and xylem loading from that of root cortex cells is in agreement with the observation that selective inhibitors of protein synthesis strongly impair xylem loading of nutrients, such as K, without affecting their accumulation in the roots and that diurnal fluctuations in nutrient uptake by the roots and their delivery to the xylem do not coincide.

6. 8.6 Pathways and fluxes

Nutrient concentrations are optimised for plant growth in hydroponic solution cultures, favourably modified in fertilised agricultural soils where phosphorus and nitrogen status and pH might be adjusted, and totally unmodified in natural ecosystems. Most essential nutrients for plant growth are present in soil solution at concentrations well below those found in plant tissues (e.g. phosphorus, potassium and nitrogen) while other ions can be in excess around roots (e.g. boron, aluminium and sodium). Plants nevertheless colonise all these environments and produce biomass at impressive rates by controlling water and ion influx. The sensitivity with which roots recognise ions in soil solution is critical to plant survival. For example, exclusion of undesirable ionic species by root membranes will leave ions relatively harmlessly in the rhizosphere whereas passage of these ions to shoots will have more dire outcomes such as leaf abscission and necrosis. Equally, membranes allow root cells to absorb essential ions selectively, even when they are chemically similar to deleterious ions. Water and ions move through root tissues along either a symplasmic pathway (intracellular), an apoplastic pathway (extracellular) or a transcellular path-way involving passage through the tonoplast membranes of vacuoles (Fig.24). While each route is explicitly defined, it is, in practice, technically difficult to determine flow rates along each pathway.

8.6. ábra - Figure 24.
By definition, non-apoplastic flow requires transport across membranes but the intracellular distance traversed and number of membranes crossed when ions travel through cells is variable. Water and ions can move through a series of plasmodesmal connections, thereby remaining in the cytoplasm until reaching the stele. Conductivity in this case is largely regulated by plasmodesmal resistance. Alternatively, water and some ions enter vacuoles and are therefore subject to transport properties of the tonoplast (‘transcellular flow’). Ultimately, most water and ions enter the apoplasms when released into mature xylem vessels, either from xylem parenchyma cells or after rupture of immature xylem elements. Alternatively, flow across the cortex might be largely apoplastic as water and ions are drawn through intercellular spaces and cell walls up to the endodermis, where they generally enter the symplasm. Concentrations of ions in the rhizosphere, transpiration rates, ionic species and membrane transport properties all have an effect on the proportion of flow through each pathway. Cells deep within the root might have a lower capacity for active uptake of ions into the symplasm than outer cell layers but can none the less absorb K+ when concentrations are high. Entry of anions to deep layers of the cortex is likely to be restricted by charge repulsion from dissociated, negative carboxyl groups in cell walls (Donnan Free Space). In general, cations also pass through cell walls more slowly than through solutions, particularly if many of the carboxyl groups in cell walls are not occupied by Ca2+ ions. None the less, apoplastic flow of water through roots can sustain large ion fluxes during periods of high transpiration. Estimates of net flux of water and ions do not reveal the absolute rates of influx and efflux: there is evidence for leakage of many ions (e.g. nitrate and orthophosphate) out of root cells and water can also cross membranes bidirectionally when water potential gradients favour water loss from roots in very dry soil. The case for efflux of orthophosphate, nitrate and sulphate has been made particularly convincingly with evidence that minimum ion concentrations extracted by roots are largely determined by efflux rates. Downregulating efflux of an ion allows roots to extract that ion to a lower concentration. Electrochemical gradients are not the only factors in ion efflux: ion-specific channels and carrier proteins in membranes can confer genetic control on efflux rates. Outwardly directed K+ channels and Na+ efflux pumps are two membrane transport proteins likely to play an important role in efflux.

Phloem and xylem are complex tissues that perform transportation of food and water in a plant. They are the vascular tissues of the plant and together form vascular bundles. They work together as a unit to bring about effective transportation of food, nutrients, minerals and water.

8.1. táblázat - Table 2. The main differences between phloem and xylem transport

<table>
<thead>
<tr>
<th></th>
<th>Phloem</th>
<th>Xylem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Function:</td>
<td>Transportation of food and nutrients such as sugar and amino acids from leaves to storage organs and growing parts of plant. This movement of substances is called translocation.</td>
<td>Water and mineral transport from roots to aerial parts of the plant.</td>
</tr>
<tr>
<td>Movement:</td>
<td>Bidirectional (Moves up or down the plant's stem from &quot;source to sink&quot;)</td>
<td>Unidirectional (Moves up the plant's stem)</td>
</tr>
<tr>
<td>Occurrence:</td>
<td>Roots, stems and leaves. transporst sucrose to growth (roots and shoots) and storage regions of the plant (seeds fruit and swollen roots)</td>
<td>Roots, stems and leaves</td>
</tr>
<tr>
<td>Additional Functions:</td>
<td>Forms vascular bundles with xylem</td>
<td>Forms vascular bundles with phloem and gives mechanical strength to plant due to presence of lignin cells. The lignified secondary wall also</td>
</tr>
</tbody>
</table>
### Phloem | Xylem
--- | ---
**Structure:** Elongated, tubular shape with thin walled sieve tubes. The sieve tubes have pores at each end in the cross walls and microtubules that extend between sieve elements allowing longitudinal flow of material. | Tubular shape with no cross walls which allows a continuous column of water + facilitates more rapid transport within the xylem vessels. There are two types - protoxylem (first formed xylem) + metaxylem (mature xylem) depending on pattern of lignin.

**Elements:** Sieve tubes, companion cells, phloem parenchyma (loosely packed resulting in intercellular spaces which allows gas exchange), bast fibers, intermediary cells. | Tracheids, vessel elements, xylem parenchyma (loosely packed resulting in intercellular spaces which allows gas exchange), xylem sclerenchyma.

**Nature of tissue:** Living tissue with little cytoplasm but no nucleus/tonoplast. | Dead tissue at maturity so it is hollow with no cell contents.

**Shape:** Phloem is not star shaped. | Xylem is star shaped.

**Location in vascular bundle:** Phloem occur on outer side of the vascular bundle. | Xylem occupy the center of the vascular bundle.

#### 8.7 ábra - Figure 25. The transport in xylem and phloem

![Figure 25. The transport in xylem and phloem](image)

#### 7. 8.7 Literature


8. Acquisition of nutrients, membrane and long way transport


8. 8.8 Questions

1. What are channel proteins and what role do they play in nutrient uptake?
2. Describe the structure of biological membrane!
3. What do the following expressions mean: uniport, antiport, symport?
4. What is the difference between apoplast and symplast pathway?

The beneficial effect of adding mineral elements (e.g., plant ash or lime) to soils to improve plant growth has been known in agriculture for more than 2,000 years. Nevertheless, even 150 years ago it was still a matter of scientific controversy as to whether mineral elements function as nutrients for plant growth. It was mainly to the credit of Justus von Liebig (1803–1873) that the scattered information concerning the importance of certain elements for plant growth was compiled and summarized and that the mineral nutrition of plants was established as a scientific discipline. These achievements led to a rapid increase in the use of mineral fertilizers. By the end of the nineteenth century, especially in Europe, large amounts of potash, superphosphate and, later, inorganic N were used in agriculture and horticulture to improve crop growth and production.

Liebig’s conclusion that the elements N, S, P, K, Ca, Mg, Si, Na and Fe are essential for plant growth was reached by observation and speculation rather than by precise experimentation. The fact that the ‘mineral element theory’ was based on this unsound foundation was one of the reasons for the large number of studies undertaken at the end of the nineteenth century. From these and other extensive investigations on the elemental composition of different plant species growing on various soils, it was realized as early as the beginning of the last century that neither the presence nor the concentration of an element in a plant is a criterion for essentiality. Plants have a limited capability for the selective uptake of those elements, which are essential for their growth. Additionally they take up elements which are not needed for growth and which may even be toxic.

The term essential mineral element (or mineral nutrient) was proposed by Arnon and Stout (1939). These authors concluded that, for an element to be considered essential, three criteria must be met:

1. A given plant must be unable to complete its lifecycle in the absence of the element.
2. The function of the element must not be replaceable by another element.
3. The element must be directly involved in plant metabolism – for example, as a component of an essential plant constituent such as an enzyme – or it must be required for a distinct metabolic step such as an enzyme reaction.

The mineral nutrients are divided into two groups: macronutrients and micronutrients

Macronutrients can be broken into two more groups: primary and secondary nutrients.

The primary nutrients are nitrogen (N), phosphorus (P), and potassium (K). These major nutrients usually are lacking from the soil first because plants use large amounts for their growth and survival.

The secondary nutrients are calcium (Ca), magnesium (Mg), and sulfur (S). There are usually enough of these nutrients in the soil so fertilization is not always needed. In addition, large amounts of Calcium and Magnesium are added when lime is applied to acidic soils. Sulphur is usually found in sufficient amounts from the slow decomposition of soil organic matter, an important reason for not throwing out grass clippings and leaves.

Micronutrients are those elements essential for plant growth, which are needed in only very small (micro) quantities. These elements are sometimes called minor elements or trace elements, but use of the term micronutrient is encouraged by the American Society of Agronomy and the Soil Science Society of America. The micronutrients are boron (B), copper (Cu), iron (Fe), chloride (Cl), manganese (Mn), molybdenum (Mo) and zinc (Zn). Recycling organic matter such as grass clippings and tree leaves is an excellent way of providing micronutrients (as well as macronutrients) to growing plants.

1. 9.1 Sulphor

1.1. 9.1.1 Sulphate Uptake, Assimilation and Reduction
Sulphate uptake into root cells is a high affinity H+ cotransport with the expression of the respective genes being strongly induced by S deficiency. Lower affinity transporters from the same gene family are involved in cell to cell distribution of sulphate across plasma membranes, and in storage and remobilization from vacuoles across the tonoplast. The transporters for delivery of sulphate into the chloroplasts, the site of activation and reduction, remain unknown. In higher plants and in green algae, the first step of S assimilation is the activation of the sulphate ion by ATP (Fig. 26). In this reaction the enzyme ATP sulphurylase catalyses the replacement of two phosphate groups of the ATP by the sulphuryl group, which leads to the formation of adenosine phosphosulphate (APS) and pyrophosphate (Fig. 26).

9.1. ábra - Figure 26. Regulation of metabolism by disulfide ↔ dithiol interchange

The diagram shows how thioredoxin functions as a regulation factor through reduction of a regulatory disulfide on a target enzyme. In the case of C assimilation, the source of electrons for thioredoxin reduction is Fd, reduced via the light reactions of photosynthesis. Thioredoxins also exist in the cytoplasm of plants where NADPH + H+ serve as an electron source. Recent evidence shows that thioredoxin has the potential to act as an oxidant mediating the formation of a disulfide bond on a target enzyme. This activity could be important for activation of antioxidant enzymes during oxidative stress.

This enzyme is regulated by various external (e.g., light) and internal (e.g., reduced sulphur compounds) factors. The activated sulphate, adenosine phosphosulphate (APS), can serve as substrate for the synthesis of sulphate esters or sulphate reduction. For the synthesis of sulphate esters such as sulpholipids, the enzyme APS kinase catalyses the formation of phosphoadenine phosphosulphate (PAPS) in an ATP-dependent reaction (Fig. 27). From PAPS, the activated sulphate can be transferred to a hydroxyl group forming a sulphate ester.

For sulphate reduction, the activated sulphate of APS is reduced to sulphite (SO32+) by APS reductase (sometimes called APS sulphotransferase) requiring two electrons supplied from glutathione. Subsequently, six electrons from ferredoxin are required to produce sulphide (S2+), catalysed by sulphite reductase, the sole reaction of the pathway which only occurs in the chloroplast. The newly formed sulphide is transferred to O-acetylserine, by the enzyme O-acetylserine (thiol) lyase (OASTL). The substrate O-acetylserine is synthesized from serine and acetyl CoA catalysed by serine acetyl transferase (SAT). However, this enzyme is only active when it occurs in a complex with OASTL (in contrast, OASTL is inactive in the complexed state). Excess O-acetylserine (occurring when sulphide is limiting) disrupts the complex, resulting in inactive SAT, and limiting further O-acetylserine production and consumption of acetyl CoA. In addition, O-acetylserine is thought to be part of a signalling pathway which stimulates expression of genes for the transporters and APS reductase to enhance sulphate acquisition and S flux to sulphide. Such positive regulation of expression may balance an apparent repression of gene expression of the sulphate transporters and APS reductase caused by reduced S compounds. Cysteine, the first stable product of the assimilatory SO42+ reduction, acts as a precursor for the synthesis of all other organic compounds containing reduced S including glutathione and methionine, as well as for other biosynthetic pathways, such as the formation of ethylene.

9.2. ábra - Figure 27.
9. The function of nutrients in plant physiological processes, deficiency symptoms I.

Plant sulfur assimilation pathways showing only those enzymes that have been conclusively demonstrated. The top line shows $\text{SO}_4^{2-}$ activation and reduction. The sulfation pathway is shown in the second line on the left and assimilation of reduced sulfur into Cys on the second line on the right. All enzymes are shown in bold above the reaction arrow, whereas intermediates are shown below the chemical structure or in isolation when the chemical structure is not shown. The R-group in sulfated metabolite refers to the metabolite that is sulfated. $\text{Fd}$ indicates the reduced and oxidized forms of Fd.

Sulphate uptake and assimilatory reduction are regulated at various levels by:

- regulation of expression of the sulphate transporters,
- modulation of the activity of ATP sulphurylase,
- the availability of sulphate as a substrate for ATP sulphurylase,
- change in the level of APS reductase expression and activity, and
- the state of complexation of SAT and O-acetylserine(thiol)lyase which may act as both a sensor (of plant S nutritional status) and a regulator (of cysteine biosynthesis).

At high cellular concentrations of either cysteine or SO2 the evolution of hydrogen sulphide (H2S) from green cells is strongly enhanced by light. The light-dependent SO2 reduction coupled with H2S release from green leaves is considered an important mechanism for the detoxification of SO2 in leaves and needles. This type of sulphate reduction may be considered a modification of the dissimilatory sulphate reduction pathway in prokaryotic anaerobes such as Desulfovibrio, which use sulphate as an oxidant in the formation of ATP and sulphide during respiration.

In higher plants the isoforms of enzymes of the assimilatory sulphate reduction pathway occur in various subcellular compartments in both leaves and in roots. In many, but not all, C4 plants, the bundle sheath chloroplasts are the main sites of sulphate assimilation, whereas the mesophyll chloroplasts are the sites of nitrate assimilation. Mesophyll chloroplasts, however, do contain at least sulphite reductase and cysteine synthase. Glutathione biosynthesis occurs in both cell types.

In general, sulphate reduction is several times higher in green leaves than in roots, and in leaves, the reaction is strongly stimulated by light. This light enhancement is to be expected because of the requirement for glutathione and ferredoxin as reductants for APS and sulphite, respectively. In addition, expression of several of the genes for enzymes of the reductive assimilation pathway (e.g., genes encoding for ATPS, APR, SiR and OASTL) appear to be under light and/or diurnal regulation. The stimulation of sulphate reduction by light may also be related to higher levels of serine synthesized during photorespiration. Reduced sulphur compounds, mainly glutathione, are exported from the leaves via the phloem to sites of demand for protein synthesis (e.g., in the shoot apex, fruits, but also roots) and may also be involved in regulation of sulphate uptake by roots. During leaf
development, the pattern of sulphate reduction is similar to that of nitrate reduction; that is, it is maximal during leaf expansion, but declines rapidly after leaf maturation. Compared with nitrate reduction, the reduction of sulphate seems to be under a strict negative feedback control as high concentrations of reduced sulphur compounds are rare. Secondary plant products are an exception.

1.2. 9.1.2 Metabolic Functions of S

Sulphur is a constituent of the amino acids cysteine and methionine, and hence of proteins. Both amino acids are precursors of other S-containing compounds such as coenzymes and secondary plant products. Sulphur is a structural constituent of these compounds (e.g., R1-C-S-C-R2) or acts as a functional group (e.g., R-SH) directly involved in metabolic reactions. About 2% of the organically reduced S in plants is present in the water-soluble thiol (-SH) fraction, and under normal conditions the tripeptide glutathione accounts for more than 90% of this fraction. Glutathione has many functions in plants and its roles in metabolism have been extensively studied. The synthesis of glutathione occurs in two steps. In the first step, glutacysteine is produced from glutamate and cysteine. In the second step, glycine is coupled to glutamylcysteine, mediated by glutathione synthase, an enzyme which requires Mg for activity. In some legume species in the second step, alanine rather than glycine is used by glutathione synthase, forming homo-glutathione, which functions similarly to glutathione.

In plants, the glutathione concentration is usually higher in leaves than in roots, and in leaves more than 50% of it is localized in the chloroplasts where it may reach millimolar concentrations. Also in root apical zones, for example of maize, the glutathione concentration is in the range of 0.7 mmol kg⁻¹ fw, about four times higher than that of cysteine. Glutathione is readily water soluble and a powerful antioxidant in plants, probably of much greater importance than the cysteine - cystine redox system. Particularly in the chloroplasts, the antioxidants glutathione and ascorbate play a key role in detoxification of oxygen radicals and hydrogen peroxide, for example in the ascorbate peroxidase–glutathione reductase cycle. In the cells, glutathione is maintained in its reduced form by the enzyme glutathione reductase. The antioxidative role of glutathione is reflected, for example, in the increase in glutathione reductase activity at high light intensities in Mg-deficient plants, or in response to other oxidative stresses such as ozone or sulphur dioxide. Conjugation of reduced glutathione to a number of xenobiotics such as atrazine (used for weed control) is also the mechanism of detoxification and, thus, of resistance of some plant species to certain xenobiotics.

Glutathione may function as a transient storage pool of reduced S and thereby maintain a certain cellular cysteine concentration. Glutathione is also the precursor of phytochelatins, which are important in detoxifying certain heavy metals in higher plants. Plant cells respond to exposure to high concentrations of heavy metals such as Cu, Cd and Zn, by increasing the synthesis of phytochelatins, and additionally, the synthesis of cysteine-rich polypeptides (metallothioneins).

9.3. ábra - Figure 28. Methionine – cysteine metabolic pathways
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(1), Transmethylation; (2), trans-sulfuration; (3), folate-dependent remethylation; (4), folate-independent remethylation; B6, vitamin B6; B12, vitamin B12; BHMT, betaine homocysteine methyltransferase; 5-CH3 THF, 5-methyltetrahydrofolate; DMG, dimethylglycine; MS, methionine synthase; MT, methyltransferase; 5,10-MTHF, 5,10-methyltetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate (Tesseraud et al., Role of sulfur amoni acids in controlling nutrient metabolism and cell functions: implications for nutrition. In: British Journal of Nutrition 101, 1132-1139).

1.3. 9.1.3 S Supply, Plant Growth and Plant Composition

Sulphur requirement for optimal growth varies between 0.1 and 0.5 % of the dry weight of plants. For the families of crop plants, the requirement increases in the order Gramineae < Leguminosae < Cruciferae and this is also reflected in corresponding differences in the S concentration (g kg⁻¹) of their seeds: 1.8–1.9, 2.5–3.0 and 11–17, respectively. The protein S concentration also varies considerably both between the protein fractions of individual cells and among plant species. On average, proteins from legumes contain less S than proteins from cereals, the N/S ratios being 40:1 and 30:1, respectively. As with N deficiency, under S deficiency shoot growth is more reduced than root growth, leading, for example in tomato, to a decrease in shoot/root ratio from 4.4 in S-sufficient to 2.0 in S-deficient plants. Interruption of S supply decreases root hydraulic conductivity, stomatal aperture and net photosynthesis. The reduced leaf area in S deficient plants is the result of both smaller size and particularly the number of leaf cells. The number of chloroplasts per mesophyll cell may or may not be affected, for example in wheat, or strongly decreased, for example in spinach.

A drastic decrease in chlorophyll and protein concentration of leaves is a typical feature of sulphur deficiency. This is to be expected, as in leaves a high proportion of the protein is located in the chloroplasts where the chlorophyll molecules comprise prosthetic groups of the chromoprotein complex. Accordingly, under S deficiency, shortage of the S-containing amino acids cysteine and methionine not only inhibits protein synthesis but also decreases the chlorophyll concentration in leaves. In contrast, starch may accumulate as a consequence either of impaired carbohydrate metabolism at the sites of production (the source) or of low demand at the sink sites (growth inhibition).

In S-deficient plants, inhibition of protein synthesis is correlated with an accumulation of soluble organic N and nitrate. Sulphur deficiency increases the concentration of amides as well as their proportions in the soluble N fraction. The sulphate concentration is extremely low in deficient plants and increases markedly when the sulphate supply is sufficient for optimal growth. The sulphate concentration of plants is therefore a more sensitive indicator of S nutritional status than the total S concentration, the best indicators being the proportion of sulphate-S in the total S, or the ratio of sulphate to malate (which also accumulates under S deficiency).
Sulphur deficiency also leads to accumulation of the sulphate analogues, selenate and molybdenate, in plant tissues due to both decreased competition by sulphate for uptake and enhanced sulphate transporter expression.

Chlorosis is characteristic for S and of N deficiency. Unlike N, however, S is more uniformly distributed between old and new leaves and its concentration is similarly affected in old and young leaves by the level of sulphate supply. Furthermore, the distribution of S in S-deficient plants is also affected by the N supply. Sulphur deficiency symptoms may occur either in young (in combination with sufficient N) or in old (in combination with low N) leaves, indicating that the extent of remobilization and retranslocation from older leaves depends on the rate of N deficiency-induced leaf senescence, a relationship which is also found for the micronutrients Cu and Zn. In legumes, during the early stages of S deficiency, nitrogenase activity in the root nodules is more strongly reduced than photosynthesis. Symptoms of S deficiency in N2-fixing legumes are therefore indistinguishable from N-deficiency symptoms. However, in root nodules of S-deficient legumes, the bacteroids may still be well supplied with S. The high sensitivity of nitrogenase activity to S deficiency therefore reflects either impaired host plant metabolism or a direct effect on nitrogenase activity.

In S-deficient plants, not only the protein concentration decreases but also the S concentration in storage proteins, indicating that proteins with lower proportion of methionine and cysteine but higher proportions of other amino acids such as arginine and aspartate are synthesized. The decrease in S-rich proteins under S deficiency has been shown in wheat and also in other cereals and legumes. Under S deficiency in wheat, the proportion of a low-molecular-weight S-rich polypeptide decreases, and in maize, the proportion of the major storage protein zein, which has a low S concentration, increases by about 30%, whereas the proportion of the S-rich glutelin decreases by 36 to 71%. The lower S concentration of proteins influences the nutritional quality considerably: methionine is an essential amino acid in human nutrition and often a limiting factor in diets in which seeds are a major source of protein. Furthermore, a decrease in the cysteine concentration of cereal grains reduces the baking quality of flour, since disulphide bridging during dough preparation is responsible for the polymerization of the glutelin fraction. There are prospects to enhance nutritional quality of seeds, for example methionine concentration, by pathway engineering.

2. 9.2 Phosphorus

Most of the phosphate that is used in fertilizers is derived from rock phosphate, which is a non-renewable resource. Global phosphate resources are predicted to be depleted within the next 50–100 years in an era when more P fertilizers are needed to produce more food and fibre to sustain a growing global population.

Unlike nitrate and sulphate, phosphate is not reduced in plants, but remains in its highest oxidized form. Therefore, even though the more reduced oxide of phosphorus (phosphate) is sometimes advertised as a fertilizer, it is harmful when given to plants that are already short of phosphate, because it is an analogue of phosphate and inhibits its uptake. After uptake – at physiological pH mainly as H2PO4− – phosphate either remains as inorganic phosphate (Pi) or it is esterified through a hydroxyl group to a carbon chain (C-O-P) as a simple phosphate ester (e.g., sugar phosphate) or attached to another phosphate by the energy-rich pyrophosphate bond (P~(P) (e.g., in ATP). The exchange between Pi and the (P) in ester and the pyrophosphate bond is very fast. For example, Pi taken up by roots is incorporated within minutes into organic (P), but released again as Pi into the xylem. Another type of phosphate bond is the relatively stable diester (C-(P)-C). In this association phosphate forms a bridging group connecting units to more complex or macromolecular structures.

2.1. 9.2.1 P as a Structural Element

The function of phosphorus as a component of macromolecular structures is most prominent in nucleic acids, which, as components of DNA, are the carriers of genetic information and, as units of RNA, are the structures responsible for the translation of the genetic information. In both DNA and RNA, phosphate forms a bridge between ribonucleoside units to form macromolecules (Fig. 29):
Phosphate is responsible for the strongly acidic nature of nucleic acids and thus for the high cation concentrations in DNA and RNA. The proportion of P in ribonucleic acids to total organically bound P differs among tissues and cells; it is high in expanding leaves, where a large amount of ribosomal RNA is required for rapid protein synthesis, lower in mature leaves, and very low in senescing leaves.

The bridging form of P diester is also abundant in phospholipids of biomembranes. There it forms a bridge between a diglyceride and another molecule (amino acid, amine, or alcohol). In biomembranes, amine choline is often the dominant partner, forming phosphatidylcholine (lecithin) (Fig. 30):

9.5. ábra - Figure 30. Formula of phosphatidylcholine

The functions of phospholipids (and of sulpholipids) are related to their molecular structure. There is a lipophilic region (consisting of two long-chain fatty acid moieties) and a hydrophilic region in one molecule; at a lipid–water interface, the molecules are oriented so that the boundary layer is stabilized. The electrical charge of the hydrophilic region plays an important role in the interactions between biomembrane surfaces and ions in the surrounding medium. Charged ions are either attracted or repelled by the charge of the hydrophilic regions, whereas ions do not interact with the hydrophobic regions. Under P deficiency, plants may replace phospholipids by galactolipids or sulpholipids.

2.2. 9.2.2 Role in Energy Transfer

Although present in cells in relatively low concentrations, phosphate esters (C-(P)) and energy-rich phosphates ((P)~(P)) represent the metabolic energy of cells. Up to 50 esters formed from phosphate and sugars and alcohols have been identified, about 10 of which, including glucose 6-phosphate and phosphoglyceraldehyde, are most abundant. The common structure of phosphate esters is (Fig. 31):

9.6. ábra - Figure 31. Structure of phosphate ester

Most phosphate esters are intermediates in metabolic pathways of biosynthesis and degradation. Their function and formation are directly related to the energy metabolism of the cells and to energy-rich phosphates. The
energy required, for example, for biosynthesis of starch or ion uptake is supplied by an energy-rich intermediate or coenzyme, predominantly ATP (Fig. 32.).

9.7. ábra - Figure 32. Formula of AMP, ADP, ATP

![Formula of AMP, ADP, ATP](image)

Energy liberated during glycolysis, aerobic respiration, or photosynthesis is utilized for the synthesis of the energy-rich pyrophosphate bond, and upon hydrolysis of this bond ~30 kJ per mole ATP are released. This energy can be transferred with the phosphoryl group in a phosphorylation reaction to another compound, which results in the activation (priming reaction) of this compound.

ATP is the principal energy-rich phosphate required for starch synthesis. The energy-rich pyrophosphate bonds of ATP can also be transmitted to other coenzymes, which differ from ATP only in the nitrogen base, for example uridine triphosphate (UTP) and guanosine triphosphate (GTP), which are required for the synthesis of sucrose and cellulose, respectively. The activity of ATPases, mediating the hydrolysis and, thus, energy transfer, is affected by many factors, including nutrients such as Mg, Ca and K.

Liberation of PPi takes place in all of the major biosynthetic pathways, for example acylation of CoA in fatty acid synthesis, formation of APS in sulphate activation, of starch in chloroplasts, and of sucrose in the cytosol (Fig. 33).

9.8. ábra - Figure 33. Suggested pathways of starch synthesis in leaves, redrawn from Muñoz et al. (2006).
Various enzymes can make use of PPI, for example the UDP-glucosephosphorylase (Fig. x) and the proton-pumping inorganic pyrophosphatase at the tonoplast. The cellular concentrations of PPI are in the range of 100–200 nmol per gram fresh weight which similar to the range of ATP. In leaves, PPI concentrations are similar in the cytosol and stroma of chloroplasts and kept stable during the light–dark cycle.

Obviously, a very small amount of ATP satisfies the energy requirement of plant cells. For example, 1 g of rapidly metabolizing maize root tips synthesizes about 5 g ATP per day. The amounts of phospholipids and RNA are considerably higher, but these are also more stable, with a relatively low rate of synthesis.

Phosphorylation of enzyme proteins by ATP, GTP, or ADP is another mechanism by which energy-rich phosphates can modulate enzyme activities. This regulatory phosphorylation is mediated by protein kinases and can result in activation, inactivation and/or changes in the allosteric properties of the target protein. Dephosphorylation is generally a hydrolytic reaction catalysed by phosphatases. Protein phosphorylation is considered a key factor in signal transduction, for example in phytochrome-mediated responses of plants. An example of this is the light-stimulated enhancement of nitrate assimilation in leaves. PEP carboxylase is one of the key enzymes regulated by phosphorylation, in both C3 and C4 plants. In C4 plants and in CAM plants phosphorylation increases the activity of PEP carboxylase and simultaneously the enzyme becomes less sensitive to negative feedback control by high malate concentrations.

2.3. 9.2.3. P supply, Plant Growth and Plant Composition

The P requirement for optimal growth is in the range of 3 to 5 mg g⁻¹ dw during the vegetative stage of growth, but some plants that have evolved on severely P-impoverished soils contain an order of magnitude less P in their leaves. The probability of P toxicity increases at concentrations higher than 10 mg g⁻¹ dw. P toxicity in plants is rare, because plants down-regulate their Pi transporters involved in net P uptake from the root environment when supplied with more P than required for optimum growth. However, many species from severely nutrient-impoverished soils in Australia and South Africa cannot down-regulate their net P uptake and show P toxicity symptoms when fertilized with P. Some tropical food legumes are rather sensitive to P; toxicity may occur already at P concentrations in the shoot dry matter of 3–4 mg g⁻¹ in pigeon pea and 6–7 mg g⁻¹ in black gram. At the other end of the spectrum, Ptilotus polystachyus, a fast-growing non-mycorrhizal Australian native herb, accumulates P to approximately 40 mg g⁻¹ shoot dw, without signs of P toxicity.

P-starvation responses in plants are mediated via sugar signaling. Signalling of the shoot P status also involves specific microRNA molecules. In P-deficient plants, reduction in leaf expansion and also number of leaves is the most obvious effects. The average length of the cell division zone is decreased in P-deficient maize leaves, and both cell production and cell division rates are reduced. Leaf expansion is strongly related to the expansion of epidermal cells, and this process may be impaired in P-deficient plants because of a decrease in root hydraulic conductivity, due to a decreased expression of genes encoding aquaporins. In contrast to the severe inhibition of
9. The function of nutrients in plant physiological processes, deficiency symptoms I.

Leaf expansion under P deficiency, the concentrations of protein and chlorophyll per unit leaf area are less affected. The chlorophyll concentration tends to increase even under P deficiency, and P-deficient leaves have a darker green colour, because leaf expansion is more strongly inhibited than chlorophyll formation.

Compared with shoot growth, root growth is less inhibited under P deficiency, leading to a typical decrease in shoot/root ratio. This decrease in shoot/root ratio is due to the increase in partitioning of carbohydrates towards the roots, indicated by a strong increase particularly in sucrose concentration of the roots of P-deficient plants. Under P starvation, the elongation rate of individual root cells and of the roots may be enhanced. In Stylosanthes hamata, under P deficiency shoot growth declines rapidly, but roots continue to grow, not only because of reduced transport of P to the shoot, but also due to additional net translocation of P from the shoot to the roots. In certain plant species, P-deficiency-induced formation of ‘cluster’ or ‘dauciform’ root clusters is another P-starvation response. Root clusters are common on the world’s most P-impoveryished soils; they may also play an important role when a large fraction of the soil P is poorly available, because of a very high or very low pH and/or high concentrations of Fe and Al. Due to the release of carboxylates in an ‘exudative burst’ root clusters efficiently ‘mine’ P.

Despite a wide range of adaptive responses in plants to P deficiency, triggered by intricate P-starvation signalling pathways, shoot growth rate is inhibited under P limitation as is the formation of reproductive organs. Flower initiation is delayed, the number of flowers is decreased and seed formation is restricted. Premature senescence of leaves is another factor limiting seed yield in P-deficient plants. Challenges for the future, when P reserves are being depleted include the development of crops and pastures and agriculture management systems that require less P while maintaining productivity. There may well be lessons to be learned from native species that evolved in severely P-impoveryished landscapes but this remains to be explored.

3. 9.3 Magnesium

The ionic radius of Mg2+ is substantially smaller (0.065 nm) and its hydrated radius substantially larger (0.476 nm) than that of K+ and Ca2+. Thus, the volume of the hydrated Mg2+ ion is about 400 times larger than the dehydrated ion. Since ions are transported through biological membranes as dehydrated cations, Mg2+ transport proteins must possess specific features. Only recently Mg2+ transporters in higher plants have been identified. In Arabidopsis an AtMRS2/AtMGT gene family encoding Mg2+ transport proteins that are homologous to the bacterial CorA Mg2+ transporter has been described. Complementing in knockout mutants and over-expressing AtMRS2-7 in Arabidopsis enhanced growth at limiting Mg supply. These proteins are channels facilitating the transport of Mg2+ through membranes along the gradient in electro-chemical potential. The uptake of Mg2+ can be strongly depressed by other cations, such as K+, NH4+, Ca2+ and Mn2+, as well as by H+, that is, by low pH. Magnesium deficiency induced by competing cations is thus a fairly widespread phenomenon.

The functions of Mg in plants are mainly related to its capacity to interact with strongly nucleophilic ligands. The Mg ion tends to adopt octahedral coordination with a marked preference for oxygen-donor ligands or water and binds electrostatically particularly to negatively charged P groups.

The interactions of Mg2+ with proteins can be grouped into two general reaction classes:

1. Mg2+ may bind directly to a protein/enzyme and determines its structure and/or serves a catalytic role, such as central atom of the chlorophyll molecule or as bridging element for the aggregation of ribosomes;
2. Mg2+ may bind the substrate of an enzyme thus increasing the efficiency of the catalytic reaction, such as in Mg–ATP phosphorylation and the Mg-isocitrate isocytrate-lyase reaction. The specific role of Mg2+ in enzyme catalysis mainly depends on its ability to position a water molecule for participation in the catalytic reaction (outer sphere complexation). Magnesium forms ternary complexes with enzymes in which bridging cations are required for establishing a precise geometry between enzyme and substrate, for example in RuBP carboxylase. Magnesium generally binds weakly to proteins and enzymes in the cytosol, thus their activity depends on the strict control of the cytosolic free Mg2+ concentration in the range of 0.5 mM. Beyond its role in enzyme regulation, a substantial proportion of the total Mg2+ in the cell is involved in the regulation of cellular pH and the cation–anion balance.

3.1. 9.3.1 Binding Form, Compartmentation and Homeostasis
A major function of Mg in green leaves is as the central atom of the chlorophyll molecule. The proportion of total Mg bound to chlorophyll depends on Mg supply. In leaves of subterranean clover, this proportion ranges from 6 % in plants with high Mg supply to 35 % in Mg-deficient plants. Under low-light conditions, the proportion of total Mg bound in chlorophyll may even be > 50 %, for example in Mg-deficient poplar. Depending on the Mg nutritional status, between 6 and 25 % of total Mg is bound to chlorophyll. As a rule, another 5–10 % of total Mg in leaves and needles is firmly bound to pectin in cell walls or precipitated as sparingly soluble salts in the vacuole (e.g., as Mg-phosphate), and the remaining 60–90 % are extractable with water.

In cells of mature leaf tissue, ~15 % of the whole cell volume is occupied by the chloroplast, the cytoplasm and the cell wall (~5 % each), the remaining 85 % by the vacuole. Similarly to inorganic P (Pi), the concentration of Mg2+ in the ‘metabolic pool’ (i.e., in the cytoplasm and chloroplasts) has also to be strictly regulated. The concentration of Mg2+ in the metabolic pool of leaf cells is assumed to be in the range of 2–10 mM. However, the free Mg2+ (non-complexed) is expected to be lower (about 0.4 mM). As for Pi, the vacuole is also the main storage pool required for maintenance of Mg2+ homeostasis in the ‘metabolic pool’. Physiological and molecular evidence indicate that Mg2+ influx into the vacuole is mediated by an Mg2+/ H+ exchanger such as AtMHX. In needles of Mg-sufficient Norway spruce, Mg2+ concentrations in the vacuole were 13–17 mM in mesophyll cells and 16–120 mM in endodermis cells. These high concentrations function as a buffer in maintaining Mg2+ homeostasis in other cells throughout the season. In addition, vacuolar Mg2+ is also important for cation–anion balance and turgor regulation of cells.

Within the ‘metabolic pool’, the Mg2+ distribution between the cytosol and the chloroplast has to be well regulated. In isolated chloroplasts, photosynthesis is strongly inhibited even by 5 mM Mg2+ in the external solution (i.e., cytosol). This inhibition is caused by decrease in K+ influx and corresponding acidification of the stroma upon illumination. Inhibition of photosynthesis by high Mg2+ concentrations in the ‘metabolic pool’ may occur in intact plants under drought stress.

3.2. 9.3.2 Chlorophyll and Protein Synthesis

Chlorophyll and heme synthesis share a common pathway up to the level of protoporphyrin IX. The first step of chlorophyll biosynthesis, insertion of Mg2+ into the porphyrin structure is catalysed by Mg chelatase. Activation of this enzyme also requires ATP and, thus, additional Mg. Release of Mg during chlorophyll breakdown requires two steps, a chlorophyllase hydrolysing chlorophyll to chlorophyllide and phytol and Mg-dechelatase yielding Mg2+ and pheophytin.

Magnesium also has an essential function as a bridging element for the aggregation of ribosome subunits, a process that is necessary for protein synthesis. Under Mg deficiency, or in the presence of high concentrations of K+, the subunits dissociate and protein synthesis ceases. Magnesium plays a critical role in stabilizing specific conformations of nucleic acids required for their synthesis and functions, and for the activities of nucleic acid polymerases and nucleases.

Net synthesis of RNA ceased immediately in response to Mg deficiency, and synthesis resumes rapidly after the addition of Mg (Fig. 34). In contrast, protein synthesis remained unaffected for more than 5 h, but it rapidly declined thereafter. The requirement for Mg in protein synthesis was also directly demonstrated in chloroplasts. As Mg2+ readily permeates the chloroplast envelope (possibly via Mg2+ channels such as MRS2-11), a concentration of at least 0.25 to 0.40 mM Mg2+ is required in the cytosol to prevent net efflux of Mg2+ from the chloroplast and, thus, to maintain protein synthesis.

9.9. ábra - Figure 34. (A) RNA and (B) protein synthesis in Chlorella pyrenoidosa suspension culture at Mg deficiency and Mg resupply
In leaf cells, at least 25% of the total protein is localized in chloroplasts. This explains why a deficiency of Mg particularly affects the size, structure, and function of chloroplasts, including electron transfer in photosystem II. In Mg-deficient plants, Mg transport from mature to young leaves is enhanced and, thus, visual deficiency symptoms typically appear on mature leaves, indicated by enhanced rates of protein degradation, including structural proteins of the thylakoids. The breakdown of the thylakoids also explains why in Mg-deficient plants, the other plastid pigments are often similarly affected as chlorophyll. Regardless of this decline in chloroplast pigments, starch accumulates in Mg-deficient chloroplasts, which may explain the increase in dry matter of Mg-deficient leaves. Impaired export of photosynthates is another factor leading to enhanced degradation of chlorophyll in Mg-deficient source leaves.

### 3.3. 9.3.3 Enzyme Activation, Phosphorylation and Photosynthesis

There is a long list of enzymes and enzyme reactions, which require or are strongly promoted by Mg, for example glutathione synthase or PEP carboxylase. For this latter enzyme in the presence of Mg, the substrate phosphoenolpyruvate (PEP) is bound in greater quantities and more tightly. Most of the Mg-dependent reactions can be grouped into general types such as the transfer of phosphate (e.g., phosphatases and ATPases) or of carboxyl groups (e.g., carboxylase). In these reactions, Mg2+ is preferentially bound to N bases and phosphoryl groups and this is for example, the case in ATP.

The substrate for ATPases, as well as inorganic PPiases, is Mg–ATP rather than free ATP. The Mg–ATP complex is stable above pH 6, and this complex can be utilized by the active sites of ATPases for the transfer of the energy-rich phosphoryl group. An example of the Mg2+ requirement of membrane-bound ATPases. Maximal activity requires the presence of both Mg2+ and K+. In meristematic cells of Mg-sufficient roots, about 90% of the cytoplasmic ATP is complexed with Mg and the concentration of free Mg2+ is only about 0.4 mM as compared with total Mg concentrations of 3.9 mM in the tissue.

### 3.4. 9.3.4 Mg Supply, Plant Growth and Composition

The Mg requirement for optimal plant growth is 1.5–3.5 g kg⁻¹ in vegetative parts. Chlorosis of fully expanded leaves is the most obvious visible symptom of Mg deficiency. In accordance with the function of Mg in protein synthesis, Mg deficiency results in a lower proportion of protein N while the proportion of non-protein N is increased. The rate of photosynthesis per unit leaf area or unit chlorophyll is lower in leaves of Mg-deficient plants and carbohydrates accumulate (negative feedback regulation). Slight and transient Mg deficiency symptoms during the vegetative growth stage, however, do not necessarily result in low yield unless irreversible changes, such as a reduction in grain number per ear in cereals, occur. At permanently insufficient root supply, remobilization of Mg from mature leaves reduces their longevity. For example, in perennials such as Norway spruce concentrations of Mg and chlorophyll as well as rate of photosynthesis of the older needles decrease in spring when the new needles develop.

There is increasing evidence that Mg deficiency is widespread in forest ecosystems in Central Europe, exacerbated by other stress factors, in particular air pollution and soil acidification. Impairment of root growth which is also typical for declining Mg-deficient spruce stands has a considerable impact on acquisition not only of Mg but also of other nutrients and of water and, thus, on drought resistance and adaptation to nutrient-poor sites.
When Mg is deficient and the export of carbohydrates from source to sink sites is impaired, the starch concentration in storage tissues such as potato tubers and the single-grain weight of cereals decrease. In cereal grains, however, Mg may play an additional role in the regulation of starch synthesis through its effect on the concentration of Pi and phytate. As discussed above, high Pi concentrations inhibit starch synthesis. In Mg-deficient wheat grains, twice as much P remains as Pi, and there is a correspondingly smaller proportion of phytate-P, compared with the grains adequately supplied with Mg.

Increasing the Mg supply beyond the growth-limiting level results in additional Mg being stored mainly in the vacuoles, as buffer for Mg2+ homeostasis in the ‘metabolic pool’ and for charge compensation and osmoregulation in the vacuole. However, high Mg concentrations in the leaves (e.g., 15 g kg⁻¹) may be detrimental under drought stress. As the leaf water potential declines, the Mg2+ concentration in the ‘metabolic pool’ increases from 3–5 mM up to 8–13 mM in sunflower. Such high concentrations, for example in the stroma of chloroplasts, inhibit photophosphorylation and photosynthesis. In pea under drought stress, Mg2+ concentrations in the chloroplasts may increase up to 24 mM.

Generally, high Mg concentrations improve the nutritional quality of plants. For example, hypomagnesaemia (grass tetany) is a serious disorder of ruminants caused by low Mg concentrations in feed and reduced efficiency of Mg resorption. An increase in Mg concentrations of forage grasses by Mg fertilization is relatively easy to achieve. Breeding for high leaf Mg concentrations, for example in Italian ryegrass, could be an alternative. Insufficient Mg intake with the human diet leading to an Mg-deficiency syndrome has attracted considerable attention.

4. 9.4 Calcium

Calcium is a relatively large divalent cation with a hydrated ionic radius of 0.412 nm and a hydration energy of 1577 J mol⁻¹. In the apoplasm, part of the Ca is firmly bound in structures, while another part is exchangeable at the cell walls and at the exterior surface of the plasma membrane. A high amount of Ca is often sequestered in vacuoles, whereas its concentration in the cytosol is low. The mobility of Ca in the symplasm and in the phloem is also low. Most of the functions of Ca as a structural or regulatory component of macromolecules are related to its capacity for coordination, by which it provides stable but reversible molecular linkages. Calcium can be supplied at high concentrations and can reach more than 10% of the dry weight, for example in mature leaves, without symptoms of toxicity or serious inhibition of plant growth. In recent years, Ca has attracted much interest in plant physiology and molecular biology because of its role as second messenger linking environmental and developmental stimuli to their physiological responses. This role is related to perturbations in cytosolic free Ca2+ concentration.

4.1. 9.4.1 Binding Form and Compartmentation

In contrast to other macronutrients, a high proportion of the total Ca in plant tissues is often located in cell walls (apoplasm). This unique distribution is mainly the result of the large number of binding sites for Ca in the cell walls. In the middle lamella it is bound to R-COO- groups of polygalacturonic acids (pectins) in a readily exchangeable form. In dicotyledons such as sugar beet, which have a large cation-exchange capacity, and particularly when the Ca supply is low, up to 50% of the total Ca can be bound as pectates. Compared to other plant species, the Ca requirement of commelinoid monocotyledons is low which is due to their low concentration of cell wall pectate.

When Ca supply is increased, excess Ca is generally accumulated in the vacuole. Three distinct physio-types for Ca nutrition exist: ‘calcitrophes’, ‘oxalate plants’ and ‘potassium plants’, which show contrasting responses to Ca supply. Calcitrophes, such as Sedum album, contain high concentrations of water-soluble Ca complexes in their vacuoles and their accumulation of Ca is stimulated greatly by increasing Ca supply. The oxalate plants are divided into species whose vacuoles contain either soluble oxalate, such as Oxalis acetosa, or Ca-oxalate crystals, such as Silene inflata. Increasing Ca supply increases Ca accumulation in plants that precipitate Ca-oxalate, but not in plants containing soluble oxalate. Potassium plants, such as Carex pendula, contain little mineralized or water-soluble Ca and maintain a high tissue K:Ca ratio. Calcium can also be precipitated in the apoplasm as Ca-oxalate or Ca-carbonate. The shape and distribution of Ca oxalate crystals differs between plant species and has proven useful as a taxonomic character.

4.2. 9.4.2 Cell Wall Stabilization
Calcium bound as Ca-pectate in the middle lamella is essential for strengthening cell walls and plant tissues. This function of Ca is clearly reflected in the positive correlation between cation exchange capacity of cell walls and Ca concentration in plant tissues required for optimal growth. The degradation of pectates is mediated by polygalacturonase, which is strongly inhibited by high Ca concentrations. Hence, in Ca-deficient tissue polygalacturonase activity is increased, and a typical symptom of Ca deficiency is the disintegration of cell walls and the collapse of the affected tissues, such as petioles, upper parts of stems and fruits.

In leaves of plants receiving high amounts of Ca during growth, or when grown under conditions of high light intensity, a large proportion of the pectic material is in the form of Ca-pectate. This makes the tissue highly resistant to degradation by polygalacturonase. The proportion of Ca-pectate in the cell walls is also of importance for the susceptibility of the tissue to fungal and bacterial infections and for the ripening of fruits. In tomato fruit, the Ca concentration of the cell walls increases to the fully grown immature stage, but this is followed by a decline in Ca concentration and a change in its bound form just before ripening. Increasing the Ca concentration in fruits, for example, by spraying several times with Ca salts during fruit development or by post-harvest dipping in CaCl₂ solution, leads to an increase in the firmness of the fruit and delays fruit ripening.

4.3. 9.4.3 Cell Extension and Secretory Processes

In the absence of an exogenous Ca supply, root extension ceases within a few hours. This is due to impaired cell elongation, rather than lack of cell division, and is more obvious in a Ca-free nutrient solution than in distilled water, an observation consistent with the role of Ca in counterbalancing the harmful effects of high concentrations of other cations. Cell elongation in roots and shoots requires acidification of the apoplasm and replacement of Ca from the cross-links of the pectic chain, although this is only part of the process. An increase in cytosolic free Ca²⁺ concentration stimulates the synthesis of cell wall precursors and their secretion into the apoplasm. The latter process is inhibited by removing apoplastic Ca. The elongation of root hairs and pollen tubes also relies on the availability of apoplastic Ca. Calcium influx from the apoplasm is restricted to the apex of these cells and increases local cytosolic Ca²⁺ concentration, which acts as focus for the exocytosis of cell wall material and establishes a polarity for cell elongation. In root caps, the secretion of mucilage also depends on the presence of apoplastic Ca.

Callose formation is another example of a calcium-induced secretory process. Under normal conditions, cells synthesize cellulose (1.4 β-glucan units). However, in response to injury or the presence of toxic cations such as aluminium, a switch to callose (1.3 β-glucan units) production can occur. This switch is triggered by an increase in cytosolic free Ca²⁺ concentration.

Stimulation of α-amylase activity in germinating cereal seeds and the aleuron is one of the few examples of enzyme stimulation by high (millimolar) Ca concentrations. Calcium is a constituent of α-amylase, which is synthesized on the rough ER. Transport of Ca²⁺ through the ER membranes is enhanced by GA and inhibited by ABA, leading to the typical stimulation (GA) and inhibition (ABA) of α-amylase activity in aleurone cells.

4.4. 9.4.4 Membrane Stabilization

Calcium plays a fundamental role of Ca in membrane stability and cell integrity. This is evident in the increased leakage of low-molecular-weight solutes from cells of Ca-deficient tissue and, in severely deficient plants, by a general disintegration of membrane structures and loss of cell compartmentation.

Calcium stabilizes cell membranes by bridging phosphate and carboxylate groups of phospholipids and proteins. Calcium can be exchanged for other cations at these binding sites; the exchange of plasma membrane-bound Ca for Na, heavy metals, or Al can contribute to salinity, heavy metal and aluminium toxicity. To prevent indiscriminate solute leakage and influx of toxic solutes, Ca must always be present in the external solution. The membrane-stabilizing effect of Ca is most prominent under stress conditions such as freezing, low temperature and anaerobiosis. The loss of low-molecular-weight solutes, such as sugars and K ions, in response to chilling or anaerobiosis is reduced by increasing the Ca concentration in the external solution. In addition to its role in stabilizing membranes, cytosolic Ca²⁺ acts as a secondary messenger to initiate membrane repair and adaptive responses to freezing, low temperature and anaerobiosis.

4.5. 9.4.5 Cation–anion Balance and Osmoregulation

In vacuolated cells of leaves in particular, a large proportion of Ca is localized in the vacuoles, where it may contribute to the cation–anion balance by acting as a counter-ion for inorganic and organic anions. In plant
species that preferentially synthesize oxalate in response to nitrate reduction, the formation of Ca oxalate in vacuoles is important for the maintenance of a low cytosolic free Ca2+ concentration. The same holds true for plant species with preferential formation of Ca oxalate in the apoplast. The formation of sparingly soluble Ca oxalate is also important for salt accumulation in vacuoles of nitrate-fed plants without increasing the osmotic pressure in the vacuoles. Additionally, Ca plays a key role in osmoregulation through its involvement as a second messenger in the cell. Stomatal movements and nyctinastic and seismonastic movements are turgor-regulated processes induced by turgor changes in individual cells (guard cells) or tissues (e.g., motor cells of pulvini). These turgor changes are driven by fluxes mainly of K, Cl and malate as osmotically active solutes. It is now well established that a transient change of cytosolic free Ca2+ concentration is required for transduction of the signals (e.g., light, touch) to the physiological response.

4.6. 9.4.6 Ca Supply, Plant Growth, and Plant Composition

The Ca concentration of plants varies between 1 and >50 g kg-1 depending on the growing conditions, plant species and plant organ. The Ca requirement for optimum growth is much lower in monocotyledons than in dicotyledons. In well-balanced, flowing nutrient solutions with controlled pH, maximal growth rates were obtained at Ca concentrations of 2.5 (ryegrass) and 100 μM (tomato), i.e. differing by a factor of 40. This difference is mainly a reflection of the Ca demand at the tissue level, which is lower in ryegrass (0.7 mg kg-1) than in tomato (12.9 mg kg-1). Differences in Ca requirements between genotypes are closely related to Ca2+-binding sites in the cell walls, i.e. the cation-exchange capacity.

The differences between monocotyledons and dicotyledons in Ca demand shown for ryegrass and tomato have been confirmed for a large number of plant species. However, the dicotyledon Lupinus angustifolius had a Ca requirement (in terms of supply and tissue concentration) which was comparable to monocotyledons, and the growth of this species was severely depressed at higher Ca concentration in the tissue. Consequently, L. angustifolius prefers acidic soils and grows poorly in calcareous soils. Such typical calcifuge behaviour may be related to insufficient capacity for compartmentation and/or physiological inactivation of Ca.

Another factor determining the Ca requirement for optimum growth is the concentration of other cations in the external solution. Because Ca is readily replaced by other cations from its binding sites at the exterior surface of the plasma membrane, Ca requirement increases with increasing external concentrations of heavy metals, Al, Na, or protons. At low compared to high pH, the Ca2+ concentration in the external solution has to be several times higher in order to counteract the adverse effect of high H+ concentrations on root elongation. A similar relationship exists between external pH and the Ca requirement for nodulation of legumes. In order to protect roots against the adverse effects of high concentrations of various other cations in the soil solution, the Ca2+ concentrations required for optimal growth have to be substantially higher in soil solutions than in balanced flowing nutrient solutions.

An increase in the concentration of Ca2+ in the external solution often leads to an increase in the Ca concentration in the leaves, but not necessarily in low-transpiring organs such as fleshy fruits or tubers, which are supplied predominantly via the phloem. The mobility of Ca in the phloem is extremely low which can protect these organs against excessive Ca accumulation. However, high growth rates of low-transpiring organs increase the risk that tissue Ca concentration falls below the critical level required for cell wall stabilization and membrane integrity, and perhaps also its functioning as second messenger. In rapidly growing tissues, Ca deficiency-related disorders are widespread, such as tipburn in lettuce, blackheart in celery, blossom end rot in tomato or watermelon, and bitter pit in apple.

Low Ca concentrations in fleshy fruits and tubers also increase the losses caused by enhanced senescence of the tissue and by fungal infections. Even a relatively small increase in the Ca concentration of fruits can be effective in reducing or preventing economic losses caused by storage disorders.

5. 9. 5 Potassium

Potassium is a univalent cation with a hydrated ionic radius of 0.331 nm and a hydration energy of 314 J mol-1. Its uptake is highly selective and closely coupled to metabolic activity. It is characterized by high mobility in plants at all levels – within individual cells, within tissues, as well as in long-distance transport via the xylem and phloem. Uptake and transport of K+ throughout the plant is facilitated by integral membrane proteins (transporters and cation channels) which enable its movement across the plasma membrane. Potassium is the most abundant cation in the cytosol and K+ and its accompanying anions contribute substantially to the osmotic potential of cells and tissues of glycophytic plant species. For various reasons, K+ has an outstanding role in
plant–water relations. Potassium is not metabolized and it forms only weak complexes in which it is readily exchangeable. Therefore, K⁺ does not strongly compete for binding sites of divalent cations (e.g., Mg²⁺). On the other hand, due to its high concentrations in the cytosol and chloroplasts, it balances the charge of soluble (e.g., organic acid anions and inorganic anions) and insoluble anions and thus facilitates stabilizing the pH between 7 and 8 in these compartments, which is the optimum for most enzyme reactions.

### 5.1. 9.5.1 Compartmentation and Cellular Concentrations

Generally, K concentrations are maintained at 100–200 mM in the cytosol, and chloroplasts. In these compartments, it has important metabolic functions and cannot be replaced by other inorganic cations such as Na⁺. In contrast, the vacuolar K⁺ concentrations may vary between 10 and 200 mM or even reach up to 500 mM in guard cells of stomata. The functions of K⁺ in cell extension and other turgor-driven processes are related to the K⁺ concentration in the vacuoles where it can be replaced to a varying degree by other cations (Na⁺, Mg²⁺, Ca²⁺) or organic solutes (e.g., sugars). In contrast to Ca²⁺, K⁺ concentrations in the apoplast are usually low, with the exception of specialized cells or tissues (stomata, pulvini), where apoplastic K⁺ concentrations may transiently increase up to 100 mM.

For rapid uptake and transport of K⁺ throughout the plant and between different cell compartments and cells within a tissue, membrane proteins are required to facilitate movement of K⁺ through membranes. These transport proteins include high-affinity transporters and ion channels encoded by a number of genes, resulting in a large range of functional, regulatory and tissue-specific properties. Among the K⁺ channels, voltage-regulated (‘gated’) channels play a major role in the control of K⁺ influx and K⁺ efflux. Permeation rates through these channels are at least three orders of magnitude faster than those catalysed by pumps and carriers. The gating characteristics of such channels in response to environmental signals play a major role in the plant response to biotic and abiotic stresses.

Although K⁺ channels are, in principle, similar to Ca²⁺ channels, their function is different. Potassium ions act directly as solutes, changing the osmotic potential in the compartments and thereby turgor, and, as carrier of charges, also the membrane potential.

### 5.2. 9.5.2 Enzyme Activation

A large number of enzymes are either completely dependent on or are stimulated by K⁺. Potassium and other univalent cations activate enzymes by inducing conformational changes in the protein. All macromolecules are highly hydrated and stabilized by firmly bound water molecules forming an electrical double layer. Maximum suppression of this electrical double layer and optimization of the protein hydration occur at univalent salt concentrations of about 100 to 150 mM. This concentration range agrees well with the K⁺ concentrations in the cytosol and in the stroma of plants well supplied with K⁺. In general, K⁺-induced conformational changes of enzymes increase the rate of catalytic reactions, Vₘₐₓ, and in some cases also the affinity for the substrate, Km.

When bulk leaf K concentrations decrease under K deficiency, cytosolic K⁺ concentrations are maintained rather constant, whereas vacuolar K⁺ concentrations strongly decrease. However, with prolonged K deficiency, cytosolic K⁺ concentrations also decline. This has severe consequences for the activity of cytosolic enzymes, not only because of the lack of enzyme activation but also because of the inability to maintain the optimum cytosolic pH. Among the enzymes most sensitive to K deprivation are pyruvate kinase and phosphofructokinase. Based on a multi-level analysis of the response of the primary metabolism of Arabidopsis to low K supply, the primary cause of metabolic disorders in low-K plants is the direct inhibition of pyruvate kinase by low cytoplasmic K⁺ in root cells.

Potassium similarly activates starch synthase isolated from a variety of plant species and organs (e.g., leaves, seeds and tubers), with maximum activation at 50 to 100 mM K⁺. Higher concentrations, however, may be inhibitory.

Another key function of K⁺ is the activation of membrane-bound proton-pumping ATPases. This activation not only facilitates the transport of K⁺ from the external solution across the plasma membrane into the root cells, but also makes K the most important element in cell extension and osmoregulation. Potassium also specifically activates vacuolar (tonoplast) pyrophosphatase isoforms involved in the transport of H⁺ into the vacuoles. Potassium deficiency increases the activity of certain hydrolases or oxidases such as polyphenol oxidase.

These changes in enzyme activities in K-deficient plant tissues lead to typical changes in the metabolite pattern: an increase in soluble carbohydrates, particularly reducing sugars, and soluble organic N compounds,
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particularly N-rich and positively charged amino acids, whereas the concentrations of nitrate, organic acids, negatively charged amino acids and pyruvate are decreased.

It is not clear to which degree these changes in enzyme activities are caused by direct or indirect effects of K+ on enzyme activity. An indirect effect may be the role of K+ in maintaining the cytosolic pH and the anion–cation charge balance. An instructive example of indirect effects is the accumulation of the diamine putrescine in K-deficient plants by a factor of 80–100. The enzymes which catalyse the synthesis of putrescine from arginine via agmatine are inhibited by high K+ concentrations and stimulated by low cellular pH. Putrescine, a divalent cation, can replace K+ in maintenance of high cytoplasmic pH; in K-deficient plants, putrescine concentrations may account for up to 30% of the deficit in K+ equivalents. In agreement with this compensatory function of putrescine, external supply of putrescine to K-deficient plants enhanced growth and prevented visual symptoms of K deficiency.

Potassium deficiency alters assimilate partitioning and thus changes metabolite concentrations in vegetative plant organs. Accumulation of sugars in mature leaves is the consequence of inhibited export from the leaves and a lower demand by sink organs such as growing leaves and fleshy fruits such as tomato.

5.3. 9.5.3 Protein Synthesis

Potassium is required in higher concentrations for protein synthesis than for enzyme activation, which is maximal already at about 50 mM K+. In cell-free systems, the rate of protein synthesis by ribosomes isolated from wheat germ is optimal at 130 mM K+ and ~2 mM Mg2+. It has been suggested that K+ is involved in several steps of the translation process, including the binding of tRNA to ribosomes. In green leaves, the chloroplasts account for about half of both leaf RNA and leaf protein. In C3 species, the majority of the chloroplast protein is RuBP carboxylase. Accordingly, the synthesis of this enzyme is particularly impaired under K deficiency and responds rapidly to resupply of K. Maximum activation was obtained at 10 mM K+ in the external solution. This concentration must have been sufficient to obtain a more than 10-fold higher K+ concentration in the chloroplasts which is required for high rates of protein synthesis.

The role of K in protein synthesis is not only reflected in the accumulation of soluble N compounds (e.g., amino acids, amides and nitrate) in K-deficient plants but can also be demonstrated directly through incorporation of 15N-labelled inorganic N into the protein fraction. K+ not only activates nitrate reductase, but is also required for the synthesis of this enzyme.

5.4. 9.5.4 Photosynthesis

Photosynthesis is strongly reduced in K-deficient leaves. Potassium affects photosynthesis at various levels. The K nutritional status affects photosynthesis via its function in stomatal regulation with K deficiency increasing stomatal resistance to CO2. However, the higher leaf internal CO2 concentration clearly shows that the leaf mesophyll resistance is more important than stomatal resistance in limiting photosynthesis in K-deficient leaves.

Potassium is the dominant counter-ion to the light-induced H+ flux across the thylakoid membranes and for the establishment of the transmembrane pH gradient necessary for the synthesis of ATP (photophosphorylation), in analogy to ATP synthesis in mitochondria.

The role of K in CO2 fixation has been most clearly demonstrated with isolated chloroplasts. An increase in the external K+ concentration to 100 mM, which is equivalent to the K+ concentration in the cytosol of intact cells, stimulated CO2 fixation more than three-fold. Upon illumination, additional influx of K+ from the cytosol is required for the maintenance of a high pH in the stroma necessary for optimal RuBP carboxylase activity. This additional influx is mediated by an H+/K+ counterflow through the chloroplast envelope. For maximum H+-ATPase activity, an external K+ concentration of about 100 mM is necessary.

With decreasing leaf K concentration, not only the rate of photosynthesis and RuBP carboxylase activity, but also photosrespiration is decreased. This may be due to a depletion of CO2 at the catalytic sites of the enzyme. On the other hand, dark respiration increases. Higher respiration rates are a typical feature of K deficiency and may reflect the higher substrate (sugars) availability for respiration.

5.5. 9.5.5 Osmoregulation

The role of K+ in maintaining xylem-sap flow is evident from the reduced night-time stem expansion and enhanced day-time stem shrinkage in K-deficient tomato plants. In principle, at the level of individual cells or in
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Potassium, as the most prominent inorganic solute, plays a key role in these processes.

5.6. 9.5.6 Cell Extension

Cell extension involves the formation of a large central vacuole occupying 80–90% of the cell volume.

There are three major requirements for cell extension:

1. cell extensibility (rearrangement or loosening of the existing cell wall),
2. synthesis and deposition of newly formed wall components,
3. solute accumulation to create the necessary internal osmotic potential for turgor pressure. In most cases, cell extension is due to K+ accumulation in the cells, which is required for both stabilizing the pH in the apoplast and the cytoplasm and increasing the osmotic potential in the vacuoles. A decrease in apoplastic pH is necessary to activate enzymes involved in cell wall loosening. Potassium is required to electrochemically counterbalance the ATPase-driven H+ release into the cell wall. In Avena coleoptiles, IAA-stimulated H+ efflux was electrochemically balanced by a stoichiometric K+ influx; in the absence of external K+, IAA-induced elongation declined and ceased after a few hours.

Potassium associated with either inorganic anions or organic acid anions is the main solute required in the vacuoles for turgor-driven cell extension. Thus, cell extension not only in leaves but also in roots is positively correlated with their K concentration. Potassium deficiency significantly reduced turgor, cell size and leaf area in expanding leaves of bean plants. Reduced leaf extension rate was a most sensitive indicator of K deficiency in maize grown in the field and under controlled conditions in hydroponics. This inverse relationship between K concentration in plants and cell size also holds true for storage tissues such as carrot and tomato.

The stimulation of stem elongation by gibberellic acid (GA) is also dependent on K supply. Potassium and GA act synergistically, the highest elongation rate being obtained when both GA and K are applied. Furthermore, the results indicate that K+ and reducing sugars act in a complementary manner to produce the turgor potential required for cell extension. At low K supply, however, GA-stimulated growth was correlated with a marked increase in K+ concentration in the elongation zone to a level similar to that of the reducing sugars. As K+ was supplied together with Cl- (as KCl), a substantial proportion of the effects on plant growth and sugar concentrations may be due to the combined effects of K+ and Cl-on osmotic potential.

The extent to which sugars and other low-molecular-weight organic solutes contribute to the osmotic potential and turgor-driven cell expansion depends on the K nutritional status of plants, as well as on plant species and specific organs. For example, in the elongation zone of leaf blades of tall fescue, about half of the imported sugars are used for accumulation of osmotically active fructanes in the vacuoles.

After completion of cell extension, K+ can be fairly readily replaced for maintenance of the cell turgor in the vacuoles by other solutes such as Na+ or reducing sugars. At later stages of leaf extension, sugars even overcompensated leaf-tissue K+ deficiency in cotton. Generally, there is a negative relationship between tissue concentrations of K+ and sugars, reducing sugars in particular which can also be observed during the growth of storage tissues. The osmotic potential of the press sap from the storage root of carrot remains constant throughout growth. Before sugar storage begins, K+ and organic acids are the dominant osmotic substances. During sugar storage, however, an increase in the concentration of reducing sugars is compensated for by a corresponding decrease in the concentration of K+ and organic acid anions. In storage roots of sugar beet, the same holds true for the concentrations of sucrose and K+.

5.7. 9.5.7 Stomata Movement

In most plant species, K+, associated with an anion, plays a major role in turgor changes in the guard cells during stomata movement. Increasing K+ concentration in the guard cells increases their osmotic pressure and results in the uptake of water from the adjacent cells, which results in an increase in turgor in the guard cells and thus stomata opening for faba bean. The accumulation of K+ in guard cells of open stomata can also be shown by X-ray microprobe analysis. Closure of the stomata in the dark is correlated with K+ efflux and a corresponding decrease in the osmotic pressure of the guard cells.

The metabolic and transport systems involved in stomata opening are shown schematically in Fig. 35.
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9.10. ábra - Figure 35. Stomata movement controlled by potassium

9.11. ábra - Figure 36. The transport of Cl- and K+ to the guard cells
At low Cl\textsuperscript{−} availability, or in plant species, which do not use Cl\textsuperscript{−} as accompanying anion for K\textsuperscript{+} in guard cells, the H\textsuperscript{+}\textendash driven K\textsuperscript{+} influx activates PEP carboxylase in the cytoplasm. The synthesized malate\textsuperscript{2+} in the guard cell cytosol is transported into the vacuole through anion channels and/ or a malate carrier and serves as accompanying anion for K\textsuperscript{+} in the vacuole. The C\textsubscript{3} compound phosphoenolpyruvate (PEP) required for malate synthesis is supplied primarily via starch degradation in the guard cell chloroplasts. In plant species such as onion, which lack starch in the guard cell chloroplasts, on the other hand, Cl\textsuperscript{−} is the main counter-ion for K\textsuperscript{+} influx, at least for stomatal regulation.

Sugars have been discussed as alternative osmotic solutes for stomatal opening. Sugars may be produced directly through photosynthesis or starch degradation in the guard-cell chloroplasts or derive from uptake from the apoplast of sugars released into the guard-cell apoplast by mesophyll cells. They are taken up and loaded into the vacuole by sugar transporters. However, the rate of sugar uptake and production in guard cells is insufficient to meet the high requirement for rapid stomatal opening. Nevertheless, sugars may be important for the sustained opening of the stomata, and particularly under K deficiency sugars may contribute substantially to osmoregulation in guard cells.

Closure of the stomata is induced by darkness, dehydration and ABA, and is associated with rapid efflux of K\textsuperscript{+} and accompanying anions from the guard cells. Whereas stomata opening is based on active transport, closure is due to the release of solutes along their concentration gradients via channels. Stomatal closure is associated with a strong increase in K\textsuperscript{+} and Cl\textsuperscript{−} concentrations in the apoplast of guard cells; for example, in Commelina communis, from 3 mM K\textsuperscript{+} and 4.8 mM Cl\textsuperscript{−} in open stomata to 100 mM K\textsuperscript{+} and 33 mM Cl\textsuperscript{−} in closed stomata. In roots and shoots of angiosperm parasites such as Striga and Loranthus, stomata remain open permanently and do not respond to darkness, ABA or drought stress. This anomalous behaviour is caused by exceptionally high K\textsuperscript{+} concentrations in the leaves of these parasites (which lack a phloem) and the lack of the capability of release of K\textsuperscript{+} from the guard cells, required for stomata closure.

Dark-induced stomata closure is initiated by a strong depolarization of the vacuolar and plasma membrane which activates the outward-rectifying K\textsuperscript{+} and anion channels. The membrane depolarization is triggered by the cessation of the ‘blue light’ activation of H\textsuperscript{+}\textendash ATPases and the ‘red light’-dependent CO\textsubscript{2} assimilation giving rise to elevated intracellular CO\textsubscript{2} concentrations.

The induction of stomatal closure by ABA derives from the roots via the xylem as ‘non-hydraulic’ signal. However, endogenous ABA from guard cells may also serve this function; ABA concentrations in the guard cells are in the range of 2.5 mM compared with about 0.9 mM in other epidermal cells in faba bean. ABA-induced stomatal closure is triggered by plasma membrane depolarization via activation of anion channels, reduced H\textsuperscript{+}\textendash ATPase activity and an increase in cytoplasmic Ca\textsuperscript{2+} concentration through stimulation of Ca\textsuperscript{2+} channels.

It is unclear how sugars are released from the vacuole and cytoplasm of guard cells. Sugars are released rather slowly upon stimuli of stomata closure, particularly under K deficiency. This slow response of sugar-loaded guard cells is presumably the reason for the ‘sluggish movement’ and incomplete opening and closure of
stomata in K-deficient plants. The incomplete closure of the stomata is responsible for the typical wilting of K-deficient plants exposed to drought stress.

6. 9.6 Literature


9. The function of nutrients in plant physiological processes, deficiency symptoms I.


7. 9.7 Questions

1. What does essentiality meant?

2. List the elements that are essential for the growth of all higher plants. Be able to identify one or more principal structural or metabolic roles for each essential element!

3. There are currently 17 elements known to be essential for higher plants. Is it possible that other elements will be added to this list in the future? Explain your answer!
10. fejezet - 10. The function of nutrients in plant physiological processes, deficiency symptoms II.

1. 10. 1. Iron

Iron is the second most abundant metal in the earth’s crust after aluminium. Solubility of Fe is, however, extremely low, especially in aerated alkaline soils. In aerated systems in the physiological pH range, the concentrations of ionic Fe3+ and Fe2+ are below 10^-15 M due to formation of Fe hydroxides, oxyhydroxides and oxides. Chelates of Fe(III) and occasionally of Fe(II) are therefore the dominant forms of soluble Fe in soil and nutrient solutions. As a rule, Fe(II) is taken up preferentially compared with Fe(III), but this also depends on the plant species (Strategies I and II). In long-distance transport in the xylem, there is a predominance of Fe(III) complexes.

As a transition element, Fe is characterized by the relative ease by which it may change its oxidation state:

\[ \text{Fe}^{3+} \leftrightarrow \text{Fe}^{2+} \]

and by its ability to form octahedral complexes with various ligands. Depending on the ligand, the redox potential of Fe(II/III) varies widely. This variability explains the importance of Fe biological redox systems. Due to the high affinity of Fe for various ligands (e.g., organic acids or inorganic phosphate) ionic Fe3+ or Fe2+ do not play a role in short- or long-distance transport in plants. In aerobic systems many low-molecular-weight iron chelates, and free iron in particular (either Fe3+ or Fe2+), produce reactive oxygen species (ROS) such as superoxide radical and hydroxyl radical and related compounds, for example:

\[ \text{O}_2 + \text{Fe}^{2+} \rightarrow \text{O}_2^- + \text{Fe}^{3+} \]

or in the Fenton reaction:

\[ \text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + \text{OH}^- + \text{OH}. \]

These radicals are highly toxic and responsible for per-oxidation of polyunsaturated fatty acids of membrane lipids and proteins. To prevent oxidative cell damage, Fe has to be either tightly bound or incorporated into structures (e.g., heme and non-heme proteins) which allow controlled reversible oxidation-reduction reactions.

1.1. 10.1.1 Fe-S Proteins

In the non-heme Fe-S proteins, Fe is coordinated to the thiol group of cysteine or to inorganic S as clusters, or to both. The most well-known Fe-S protein is ferredoxin, which acts as an electron transmitter in a number of metabolic processes according to the principle (Fig. 37):

10.1. ábra - Figure 37. The formula of ferredoxin

Details of the function of ferredoxin in these processes are discussed in the relevant sections. In Fe-deficient leaves, the concentrations of ferredoxin and chlorophyll are decreased to a similar extent with the low ferredoxin concentration correlated with lower nitrate reductase activity (NRA). Both ferredoxin concentration and NRA can be restored by resupplying Fe. Due to the involvement of Fe at various steps in nitrate reduction positive correlations between Fe supply, ferredoxin concentration and nitrate reduction are to be expected.
Another example of Fe-S proteins are the isoenzymes of superoxide dismutase (SOD) which contain Fe as a metal component of the prosthetic group (FeSOD). Superoxide dismutases detoxify superoxide anion free radicals by formation of H2O2 and may contain Cu, Zn, Mn or Fe as metal components. In chloroplasts, FeSOD is the main isoenzyme of SOD, but it may also occur in mitochondria and peroxisomes in the cytoplasm. In Fe-deficient plants, FeSOD activity is low, whereas the activity of CuZnSOD is increased, resulting in high production of H2O2. Although Fe-deficient plants have reduced levels of antioxidative enzymes such as catalase and ascorbate peroxidase and increased concentrations of H2O2, there does not appear to be enhanced oxidative cell damage (e.g., lipid peroxidation), which may be due to the very low concentrations of active Fe required for ROS generation through the Fenton reactions.

Iron deficiency stress is associated with enhanced production of organic acids, particularly citrate. Reduced aconitase activity may explain the enhanced production of organic acids in Fe-deficient plant tissues. Aconitase is an Fe-S protein which catalyses the isomerisation of citrate to isocitrate in the tricarboxylic acid cycle. Iron as metal component of the prosthetic group is required for stability and activity of the enzyme, and the Fe cluster of the enzyme is responsible for the spatial orientation of the substrates (citrate and isocitrate); valency changes are not involved in the reaction. In Fe-deficient plants, aconitase activity is lower, and reactions in the tricarboxylic acid cycle are disturbed leading to organic acids, particularly citric and malic acid. In roots of Fe-deficient Strategy I and Strategy II plants, citrate concentrations were 3.7- to 8.8-fold and 3.8- and 11.1-fold higher, respectively. Similar increases in concentration of organic acids were also found in xylem exudates and leaf apoplastic fluids of Fe-deficient plants. Such high citrate concentrations in the xylem may indicate Fe transport as stable, water soluble Fe-citrate complexes. In roots of Fe-deficient tomato plants, the increase in organic acid concentration is correlated with enhanced CO2 fixation and net excretion of H+, i.e. acidification of the rhizosphere. The relationship between lower aconitase activity and organic acid accumulation in roots of Fe-deficient plants are still a matter of controversy. Iron deficiency-induced CO2 fixation and high PEPC activity in root cells may be major reasons for accumulation of organic acids in Fe-deficient plants. Fe deficiency resulted in a 10-fold increase in PEPC activity in tomato root tip extracts which were associated with increased citrate concentration by about 20-fold in roots and 17-fold xylem sap. Increased PEPC activity may also be linked to Fe deficiency-induced root adaptive responses like proton release and Fe reduction capacity.

Recently, existence of a tri-Fe (III), tri-citrate complex (Fe3Cits) in xylem exudates of tomato plants was shown, confirming previous speculations that Fe is transported in xylem in form of Fe–citrate complex.

Riboflavin also accumulates in most dicotyledonous plant species under Fe deficiency, and its release from roots may be enhanced by a factor of 200 in Fe-deficient plants. Increased root concentrations of riboflavin are associated with the activity of 6,7-dimethyl-8-ribityllumazine synthase which contributes to the final step of riboflavin biosynthesis. Accumulation of riboflavin is presumably the result of alterations in purine metabolism due to impairment of xanthine oxidase, another enzyme with Fe-S clusters as a prosthetic group.

### 1.2. 10.1.2 Chloroplast Development and Photosynthesis

As a rule, Fe deficiency has less effect on leaf growth, cell number per unit area, or number of chloroplasts per cell than on the size of the chloroplasts and protein content per chloroplast. Iron is required for protein synthesis, and the number of ribosomes – the sites of protein synthesis – decrease in Fe-deficient leaf cells. In Fe-deficient maize leaves, for example, the total protein content decreases by 25 % but that of the chloroplasts by 82 %, most probably because of a particular high Fe requirement of chloroplastic mRNA and rRNA. In sugar beet leaves, Fe is important for RNA synthesis and a decrease in Fe concentration is associated with a strong decrease in protein synthesis. Decreases in leaf protein content under Fe deficiency are particularly pronounced for the Rubisco protein that represents nearly 50 % of the chloroplast soluble proteins.

In the thylakoid membranes, about 20 Fe atoms are directly involved in the electron transport chain. Photosystem (PS) I is a strong sink for Fe due to its higher Fe content (12 atoms of Fe per complex) compared to PS II (3 atoms of Fe per complex). The high Fe requirement for the structural and functional integrity of the thylakoid membranes, and the additional Fe requirement for ferredoxin and the biosynthesis of chlorophyll explain the particular sensitivity of chloroplasts in general, and the thylakoids in particular, to Fe deficiency. In Fe-deficient leaves, however, not all photosynthetic pigments and components of the electron transport chain are decreased to the same extent. The activity of PS I is more depressed than of PS II under Fe deficiency, probably due to a higher amount of Fe per PS I than PS II. Resupplying Fe to chlorotic leaves increases the function of PS I as an electron transmitter more strongly than that of PS II. As Fe deficiency becomes more severe, the activity of PS II also decreases and is more difficult to restore.
Under Fe deficiency, leaves generally have low photosynthetic activity due to several reasons discussed below; but they absorb more light energy per chlorophyll molecule than required for photosynthesis, especially under high radiation. This results in a high risk for photoinhibitory and photooxidative damages in Fe-deficient leaves. Nevertheless, in contrast to Zn-deficient or Mg-deficient plants, there appears to be little photooxidative damage in Fe-deficient plants. Absence of serious photooxidative damage in Fe-deficient leaves is, most probably, related to the rapid increases in levels of de-epoxizidized xanthophyll pigments and the low concentrations of catalytic Fe required in ROS generation.

Iron-deficient leaves are characterized by low concentrations of starch and sugars. This is to be expected due to the low concentrations of chlorophyll and ferredoxin, impairment of photosynthetic electron transport and the decreased regeneration of reduced ferredoxin. Reduction in photosynthesis is a characteristic physiological response of plants to Fe deficiency. Decrease in photosynthesis under Fe deficiency is attributed to reduced photosynthetic electron transport and thus impaired carboxylation due to low availability of ATP and NADPH for the Calvin cycle. The low concentration of Rubisco protein is a further important reason for the low photosynthesis in Fe-deficient plants.

1.3. 10.1.3 Localization and Binding State of Fe

When plants are grown under controlled conditions, about 80 % of the Fe is localized in the chloroplasts in rapidly growing leaves, regardless of Fe nutritional status. With Fe deficiency, a shift in the distribution of Fe occurs only within the chloroplasts, whereby the lamellar Fe concentration increases at the expense of the stroma Fe.

Iron can be stored in the stroma of plastids as phytoferritin (plant ferritin). It consists of a hollow protein shell which can store up to 5,000 atoms of iron as Fe(III) (Fe content 12–23 % dw). Its concentration is high in dark-grown leaves (up to 50% of the total Fe), but it rapidly disappears during regreening and is very low in green leaves. In young leaf tissues, ferritin-bound Fe represents an important Fe source for biosynthesis of Fe-containing proteins in photosynthesis. Ferritin is a vital compound in maintenance of Fe homeostasis and protection against oxidative damage. By sequestration of large amounts of Fe, ferritin exerts a critical protective role against peroxidative cell damage catalysed by Fe-induced formation of ROS. In plants without ferritin, ROS accumulate resulting in impairment of plant growth and development. Ferritin is not only present in chloroplasts: it can also be found in the xylem and phloem. Additionally, ferritin is abundant in seeds. In pea plants, ferritin-bound Fe represented 92 % of the total Fe in seed embryos, indicating that ferritin is probably a major form of Fe storage in seeds. However, there is a large genetic variation in seed concentration of ferritin-bound Fe among plant species. In legume species, ferritin-Fe concentration ranges from 15 % of the total Fe in kidney beans up to 69 % in lentils. During seed germination, ferritin is rapidly degraded, probably catalysed by the released Fe2+ and generation of hydroxyl radicals, which destroy the protein shell. Phytoferritin may also act as storage for Fe in nodules of legumes, for heme synthesis during nodule development and heme degradation during senescence.

Bioavailability of Fe in seeds or grains is an important issue for nutritional quality and human nutrition. Iron from ferritin in soybean and wheat seeds is bioavailable, absorbed well, and suggests that ferritin Fe is a valuable dietary source and could be a target compound for biofortification of food crops with Fe. Phytate is abundant in seeds and can bind Fe. Phytate has a high binding affinity to Fe and forms insoluble complexes with Fe. Therefore, phytate-rich diets (e.g., cereal-based foods) may be a key factor in high prevalence of Fe deficiency in humans.

If plants are grown under controlled conditions (e.g., in nutrient solutions), there is a close positive correlation between total leaf concentration of Fe and that of chlorophyll when the supply of Fe (as chelates) is suboptimal. This correlation, however, is often poor or absent in plants grown in calcareous soils where the Fe concentration in chlorotic leaves may be similar to or even higher than that in green leaves. This phenomenon has been termed ‘chlorosis paradox’. Previously, inactivation of Fe in chlorotic leaves of plants grown in calcareous soils has been discussed as a plausible explanation for the same or even higher Fe concentrations in chlorotic than green leaves. However, inactivation of Fe in leaf tissue could not be detected in later studies. Instead, the high Fe concentrations in chlorotic young leaf tissues may be the result of restricted leaf expansion growth and consequently diminished dilution of Fe concentrations by growth.

1.4. 10.1.4 Fe Deficiency and Toxicity
The critical deficiency concentration of Fe in leaves is in the range of 50–150 mg Fe kg⁻¹ dw. This refers to total Fe and is, therefore, only of limited value for characterization of the Fe nutritional status of field-grown plants. In general, C4 species require a higher Fe supply than C3 species, but their critical deficiency concentrations are similar, namely 72 mg Fe kg⁻¹ in C3 species and 66 mg Fe kg⁻¹ in C4 species. In fast growing meristematic and expanding tissues, for example shoot apices, the critical deficiency concentrations are higher, in the range of 200 mg Fev kg⁻¹ dw of total Fe. In legumes, the Fe demand for nodule development is particularly high.

Iron deficiency is a worldwide problem in crop production on calcareous soils. It is the major factor responsible for so-called lime-induced chlorosis. Iron deficiency also represents an important nutrient deficiency problem in oceans, limiting CO₂ fixation and N₂ fixation capacity of the phytoplankton.

On the other hand, Fe toxicity (‗bronzing‘) is a serious problem in crop production on waterlogged soils; it is the second-most severe yield-limiting factor in wetland rice. The critical toxicity concentrations are above 500 mg Fe kg⁻¹ leaf dw, but depend on other factors such as concentration of other nutrients. Iron toxicity may also occur under dryland conditions; drought-induced damage in photosynthetic tissue is caused by Fe-catalysed formation ROS in the chloroplasts. Iron toxicity damage is generally associated with formation of ROS, and therefore induction of antioxidative enzymes such as ascorbate peroxidase and Fe-binding proteins such as ferritin represent an important cellular defence mechanism against iron toxicity damage.

### 1.5. 10.1.5 Iron deficiency symptoms

These iron-deficient leaves show strong chlorosis at the base of the leaves with some green netting. The most common symptom for iron deficiency starts out as an interveinal chlorosis of the youngest leaves, evolves into an overall chlorosis, and ends as a totally bleached leaf. The bleached areas often develop necrotic spots. Up until the time the leaves become almost completely white they will recover upon application of iron. In the recovery phase, the veins are the first to recover as indicated by their bright green color. This distinct venial regreening observed during iron recovery is probably the most recognizable symptom in all of classical plant nutrition. Because iron has a low mobility, iron deficiency symptoms appear first on the youngest leaves. Iron deficiency is strongly associated with calcareous soils and anaerobic conditions, and it is often induced by an excess of heavy metals.

### 2. 10.2. Manganese

Manganese can exist in the oxidation states I, II, III, IV, VI and VII. In biological systems, however, it mainly occurs in oxidation states II, III and IV, with MnII and MnIV being fairly stable and MnIII unstable. In plants, MnII is by far the dominant form, but it can readily be oxidized to MnIII and MnIV. Manganese therefore plays an important role in redox processes.

The ionic radius of Mn²⁺ (0.075 nm) lies between Mg²⁺ (0.065 nm) and Ca²⁺ (0.099 nm), and it can therefore substitute, or compete with, either of these ions in various reactions. The binding strength of all three ions for ligands based on oxygen donors is quite similar or may be higher for Mn²⁺, for example, by a factor of about four in case of ATP. This has important consequences for the compartmentation of Mn²⁺ in cells and interactions between Mn and Mg.

#### 2.1. 10.2.1 Mn-containing Enzymes

Although a relatively large number of enzymes are activated by Mn²⁺, there are only a small number of Mn-containing enzymes, namely the Mn-protein in PS II, the Mn-containing superoxide dismutase (MnSOD) and oxalate oxidase. Oxalate oxidase is a secreted multimeric glycosylated Mn-containing enzyme. It is a homohexamer and belongs to a large family of germin-like proteins termed cupins because of their conserved β-barrel fold. The active site is in the centre of the β-barrel and contains a Mn ion. Initial reports on a Mn-containing purple acid phosphatase were followed by a subsequent work suggesting that this enzyme contains two Fe atoms per molecule, thus requiring Fe rather than Mn for its activity. However, more recent opinion is that both Mn-containing and Fe-containing purple acid phosphatase may exist.

Superoxide dismutases (SOD) are present in all aerobic organisms and play an essential role in the survival of these organisms in the presence of oxygen. They protect tissues from the deleterious effect of the oxygen radical O₂⁻ formed in various enzyme reactions in which a single electron is transmitted to O₂ (Fig. 38):
10. The function of nutrients in plant physiological processes, deficiency symptoms II.

10.2. ábra - Figure 38. The detoxification reaction of free radicals

\[
\begin{align*}
\text{O}_2 + e^- & \rightarrow \text{O}_2^\cdot (\text{Superoxide}) \\
\text{O}_2^\cdot + \text{O}_2^\cdot + 2\text{H}^+ & \rightarrow \text{H}_2\text{O}_2 (\text{Hydrogen peroxide}) + \text{O}_2 \\
2\text{H}_2\text{O}_2 & \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\end{align*}
\]

The SOD isoenzymes differ in their metal component, which may be Fe (FeSOD), Mn (MnSOD) or Cu + Zn (CuZnSOD). The FeSOD is mainly confined to chloroplasts. CuZnSOD is found in chloroplasts, but also occurs in the cytoplasm in peroxisomes and mitochondria. MnSOD is not widely distributed in higher plants and mainly located in mitochondria and peroxisomes. There are controversial reports concerning the occurrence of MnSOD in chloroplasts. It is absent in pea, but present in tobacco. MnSOD is present in chloroplast thylakoids of most eukaryotic algae. Numerous transgenic plants have been produced over the last two decades with MnSOD targeted to the chloroplasts; such plants showed increased tolerance to a range of abiotic stresses (e.g., salinity) and Mn deficiency. Free-living and symbiotic rhizobia (bacteroids) possess only MnSOD, whereas in the cytosol of nodules both MnSOD and CuZnSOD are present.

2.2. 10.2.2 Mn Deficiency and Toxicity

Manganese deficiency is abundant in plants growing in soils derived from parent material inherently low in Mn, and in highly leached tropical soils. It is also common in soils of high pH containing free carbonates, particularly when combined with high organic matter content.

In Mn-deficient plants, dry matter production, net photosynthesis and chlorophyll content decline rapidly, whereas rates of respiration and transpiration remain unaffected. Manganese-deficient plants are more susceptible to damage by freezing temperatures, a range of soil-borne root-rotting fungal diseases (e.g., take-all) and require twice as long to reach booting stage than Mn-sufficient plants. A decrease in grain number and grain yield in Mn-deficient plants is presumably a combination of low pollen fertility and shortage of carbohydrate supply for grain filling.

In dicotyledonous plants, intercostal chlorosis of the younger leaves is the most distinct symptom of Mn deficiency, whereas in cereals, greenish grey spots on the older leaves (‘grey speck’) are the major symptoms. In legumes, Mn-deficiency symptoms on the cotyledons are known as ‘marsh spot’ in peas or ‘split seed’ disorder in lupins; the latter disorder includes discoloration, splitting and deformity of seeds.

Manganese deficiency can be corrected by soil or foliar application of MnSO4, but the latter method has limitations. High Mn concentrations in seeds, either supplied naturally from the parent plants or artificially by soaking the seeds in MnSO4, can considerably improve plant growth and seed yield on soils with low Mn availability as has been shown for barley. In wheat, high seed Mn was more effective than Mn fertilization in achieving good yield in soils with low Mn availability. In addition, wheat produced from seed with high Mn concentration had increased tolerance to take-all disease.

Plant species and genotypes within a species differ considerably in susceptibility to Mn deficiency when grown on soils with low Mn availability. Oat, wheat, soybean and peaches are susceptible, whereas maize and rye are not. Differential Mn efficiency was reported among genotypes of bread wheat, durum wheat, barley, and other crops. Despite differences in efficiency among plant species, the critical deficiency concentrations of Mn in plants are similar, varying between 10 and 20 mg Mn kg⁻¹ dw in fully expanded leaves, regardless of plant species or cultivar or prevailing environmental conditions. Only Lupinus angustifolius has a critical deficiency concentration, which is twice as high as that of other plant species.

Of the environmental factors affecting critical toxicity concentrations, temperature and the presence of silicon are of particular importance. At high temperatures, the Mn concentration in leaves is often higher than that at low temperatures or when supplied with silicon, indicating greater tissue tolerance to Mn. In a maize genotype tolerant to Mn toxicity, silicon substantially increased the thickness of the epidermal leaf layers where excess Mn was stored. Maintaining sufficient concentrations of ascorbic acid in leaf apoplast can contribute to tolerance to Mn toxicity in cowpea and common bean cultivars, but is not a determining factor. Nevertheless, increased activity of antioxidative enzymes and increased concentrations of antioxidants contribute to...
alleviation of Mn toxicity stress in many plant species. There are conflicting views on the effect of high light intensity on Mn toxicity, with reports of increasing the severity of toxicity symptoms or lessening them. The diversity of Mn toxicity symptoms may be a major reason for these contradictory results.

In many plant species, symptoms of Mn toxicity are brown speckles on mature leaves. Although these brown speckles contain oxidized Mn, the brown colour derives not from Mn, but from oxidized polyphenols. The formation of brown speckles is preceded by enhanced callose formation in the same area, indicating toxic effects of Mn on the plasma membrane and enhanced Ca2+ influx as a signal for callose formation. The intensity of formation of brown speckles can be used as a simple and rapid method for screening different cultivars for Mn tolerance. In leaves of Mn-tolerant plant species such as sunflower or stinging nettle growing at high Mn concentrations, brown spots are also often found around the base of trichomes which contain Mn oxides and may therefore be considered as mechanism to reduce soluble Mn concentrations.

Interveinal chlorosis and necrosis are further symptoms of Mn toxicity. Particularly in dicots such as bean, soybean, cotton and blueberry, these symptoms are combined with deformations of young leaves (‘crinkle leaf’), which is a typical symptom of Ca deficiency. Hence, Mn toxicity is accompanied by induced deficiencies of other nutrients such as Ca, Mg, Fe and Zn. Induced deficiency of Fe and Mg is caused by inhibited uptake across the plasma membrane and competition (or imbalance) at the cellular level. Accordingly, Mn toxicity can often be counteracted by a high supply of Mg.

3. 10.3 Copper

Copper is a redox-active transition element with roles in photosynthesis, respiration, C and N metabolism, and protection against oxidative stress. Like Fe, it forms highly stable complexes and participates in electron transfer reactions. Divalent Cu is reduced readily to monovalent Cu, which is unstable.

Most of the functions of Cu as a plant nutrient are based on enzymatically bound Cu which catalyses redox reactions. In redox reactions of the terminal oxidases, Cu enzymes react directly with molecular oxygen. Terminal oxidation in living cells is therefore catalysed by Cu and not by Fe.

Copper has a high affinity for peptide and sulphhydryl groups, and thus to cysteine-rich proteins, as well as also for carboxylic and phenolic groups. Therefore, more than 98 % of the Cu in plants is present in complexed forms and the concentrations of free Cu2+ and Cu+ is extremely low in the cytoplasm.

3.1. 10.3.1 Cu Proteins

There are more than 100 different Cu-containing proteins in plants. About 50 % of Cu found in plants is present in chloroplasts, bound to plastocyanin, where it participates in photosynthetic reactions. Other major forms include Cu-binding chaperones and numerous enzymes, particularly single and multi Cu-containing oxidase enzymes. Copper is also part of the ethylene receptor and is involved in Mo cofactor biosynthesis. In legumes, Cu deficiency reduces nodulation and N2 fixation. Under Cu deficiency, the activity of these Cu enzymes decreases rapidly, and in most, but not all, cases, these decreases are correlated with metabolic changes and inhibition of plant growth.

3.2. 10.3.2 Carbohydrate, Lipid and N Metabolism

Due to the role of Cu in PS I, it is not surprising that Cu-deficient plants have low rates of photosynthesis and reduced carbohydrate synthesis, at least during the vegetative stage. In Cu-deficient wheat plants, the concentration of soluble carbohydrates during the vegetative stage is lower than in Cu-sufficient plants. However, when grains have developed as a dominant sink after anthesis, Cu-deficient plants produce few grains, remain green (i.e., they remain actively photosynthesizing) and have high concentrations of soluble carbohydrates in leaves and roots. The reduction in net CO2 fixation in severely Cu-deficient plants to about 50% expressed both in terms of unit chlorophyll or leaf area, cannot be attributed solely to lower activities of PS I. Lower activity of PS II must also be a contributing factor. In Cu-sufficient plants, 11 Cu atoms per 1,000 chlorophyll molecules are located in the PS II complex. Under severe Cu deficiency, polypeptides of PS II are altered and the lipid composition changes in favour of the less unsaturated fatty acids, for example 18:3→18:2.

These changes in fatty acid composition in the thylakoids and in the PS II complex are probably related to functions of Cu in the desaturation of long-chain fatty acids (e.g., 18:2→18:3).
The low carbohydrate concentrations in Cu-deficient plants can explain the impaired pollen formation and fertilization, and are the main reason for reduced nodulation and N2fixation in Cu-deficient legumes. Symptoms of N deficiency in Cu-deficient plants can be overcome by the application of mineral N. However, it has been shown that N application promotes Cu deficiency, and when N supply is high, application of Cu fertilizers may be required for maximum yield. In addition to non-specific growth enhancement by N, N affects Cu availability and mobility within the plant, including (1) a higher proportion of Cu complexed to amino acids and proteins in mature tissue and, (2) a decrease in the rate of re-translocation of Cu from old leaves to areas of new growth. Re-translocation of Cu is closely related to leaf senescence and because high N supply delays senescence, it also retards Cu re-translocation. In agreement with this, the critical deficiency concentration of Cu in the shoot required for maximum growth increases with increasing N supply.

3.3. 10.3.3. Pollen Formation and Fertilization

Copper deficiency affects grain, seed and fruit formation more strongly than vegetative growth. Supplying 0.5 μg Cu produced maximum dry weight of roots and shoots, but flower formation was impaired, and no fruits were formed. For fruit formation a much higher Cu supply was required, >1.0 μg Cu, and with 10 μg Cu, toxicity occurred.

The main reason for the decrease in the formation of generative organs is the non-viability of pollen from Cu-deficient plants. The critical stage of Cu deficiency-induced pollen sterility is microsporogenesis. Reduced seed set in Cu-deficient plants may be the result of the inhibition of pollen release, since lignification of the anther cell walls is required to rupture the stamen and release the pollen. In Cu-deficient plants, lignification of the anther cell wall is reduced or absent; the anther cell wall expands instead of supplying the developing pollen with nutrients. Following grain set in wheat and seed set in subterranean clover, further grain and seed growth, are not influenced by the Cu status of the plants, even though the Cu concentration of wheat grains in plants adequately supplied with Cu is five to six times higher than in deficient plants.

3.4. 10.3.4 Cu Deficiency

Copper deficiency is often observed in plants growing on soils either low in total Cu (e.g., ferrallitic and ferruginous coarse textured soils, or calcareous soils derived from chalk) and on soils high in organic matter where Cu is complexed with organic substances. As mentioned above, high N availability can also lead to Cu deficiency.

The critical deficiency concentration of Cu in vegetative plant parts is generally in the range of 1–5 μg g-1dw, depending on plant species, plant organ, developmental stage and N supply with the critical deficiency concentration in the youngest emerged leaf being less affected by environmental factors than that of older leaves. Plant species differ considerably in sensitivity to Cu deficiency: wheat, oats and spinach are more sensitive than, for example, pea, rye and oilseed rape. Stunted growth, distortion of young leaves, chlorosis/ necrosis starting at the apical meristem extending down the leaf margins, and bleaching of young leaves (‘white tip’ or ‘reclamation disease’ of cereals grown in organic soils), and/or ‘summer dieback’ in trees are typical visible symptoms of Cu deficiency. Enhanced formation of tillers in cereals and of auxillary shoots in dicotyledons are secondary symptoms caused by necrosis of the apical meristem. Wilting in young leaves, either also characteristic of Cu-deficient plants, is the result of impaired water transport due to insufficient lignification of the xylem vessels or of structural weaknesses in the cell wall system rather than the result of a low water content per se. The molecular responses to Cu deficiency are increased expression of metal reductases and transporters, and prioritizing Cu to essential enzymatic pathways including compensatory increases in FeSOD and MnSOD in place of CuZnSOD.

The availability of Cu can be low in many soils and this can be corrected by soil or foliar applications. Soil applications of inorganic copper as CuSO4or oxide forms, or slow-release metal compounds, sewage sludges or manures are often appropriate for long-term effects. Foliar applications of Cu in the form of inorganic salts, oxides, or chelates can be used to rapidly correct Cu deficiency in soil grown plants. The use of Cu-containing fertilizers can be used to increase the Cu concentration of the edible portions of crops where there are dietary deficiencies of Cu in humans and livestock. However, Cu fertilization must be managed appropriately since high Cu concentrations can be toxic to plants and animals. Selecting genotypes, which are highly efficient in Cu uptake, translocation from the roots to the shoots and re-translocation within the shoot, is a promising long-term approach to the prevention of Cu deficiency.

3.5. 10.3.5 Cu deficiency symptoms
These copper-deficient leaves are curled, and their petioles bend downward. Copper deficiency may be expressed as a light overall chlorosis along with the permanent loss of turgor in the young leaves. Recently matured leaves show netted, green veining with areas bleaching to a whitish gray. Some leaves develop sunken necrotic spots and have a tendency to bend downward. Trees under chronic copper deficiency develop a rosette form of growth. Leaves are small and chlorotic with splotchy necrosis.

### 3.6. 10.3.6 Cu toxicity

Toxic levels of Cu can occur under natural conditions or due to anthropogenic inputs. Anthropogenic inputs include those from the long-term use of Cu-containing fungicides (e.g., in vineyards), industrial and urban activities (air pollution, urban waste and sewage sludge), and the application of pig and poultry slurries. For most crop species, the critical toxicity level of Cu in the leaves is above 20 to 30 μg g⁻¹ dw. There are, however, marked differences in Cu tolerance between plant species. Among certain Cu-tolerant species (‘metallophytes’), particularly among the flora of the Cu-rich soils in the Democratic Republic of Congo, there have been field or herbarium reports that the Cu concentration in leaves can be as high as 1,000 μg g⁻¹ dw. However, while these species may have an elevated requirement for Cu and are certainly highly tolerant of Cu, Cu ‘hyperaccumulation’ has not been demonstrated under controlled conditions, suggesting that some of these records may be due to leaf contamination with dust.

A high Cu supply usually inhibits root growth before shoot growth. This does not mean that roots are inherently more sensitive to high Cu concentrations. With high supply, the Cu concentration of the roots increases proportionally to the concentration of Cu in the external medium, whereas transport to the shoot is still highly restricted. Critical toxicity concentrations of Cu in the shoots may therefore not necessarily reflect the Cu tolerance of plants. This is an important consideration when genotypes are compared. Even at high supply, up to 60% of the total Cu in roots can be bound to the cell wall fraction and the cell wall–plasma membrane interface.

In addition to immobilization of Cu in the root, or reductions in uptake per se through binding of extracellular Cu by root exudates, cellular mechanisms of Cu tolerance are likely to include:

- enhanced binding to cell walls,
- restricted influx through the plasma membrane,
- stimulation of efflux from the cytoplasm, including via HMA proteins,
- compartmentation of Cu by export to the vacuole,
- chelation at the cell wall–plasma membrane interface,
- intracellular chelation of Cu by organic acids, glutathione-derived phytochelatins and cysteine-rich metallothioneines in the cytoplasm.

### 4. 10.4 Zink

Zinc (Zn) is the second most abundant transition metal in living organisms after Fe. Average total Zn concentration in cultivated soils is around 65 mg kg⁻¹. Zinc is taken up predominantly as a divalent cation (Zn²⁺); at high pH, it is presumably also taken up as a monovalent cation (ZnOH⁺). In long-distance transport in the xylem, Zn either is bound to organic acids or occurs as the free divalent cation. In the phloem sap, the Zn concentrations are high, with Zn possibly complexed by low-molecular-weight organic solutes. In plants as well as in other biological systems, Zn exists only as ZnII, and does not take part in redox reactions. The metabolic functions of Zn are based on its strong tendency to form tetrahedral complexes with N-, O- and particularly S-ligands through which it plays a functional (catalytic) and a structural role in enzyme reactions. In the last decade, impressive progress has been made on identification and characterization of catalytic and structural Zn sites in proteins. Recent studies show the existence of a large number of proteins containing or binding Zn. It is estimated that up to 10% of the proteins in the human genome is proteins, which require Zn for their structural or functional activities, indicating that at least 2,800 proteins are Zn dependent. The role of Zn in protein molecules involved in DNA replication and in regulation of gene expression has attracted growing interest. Changes in metabolism induced by Zn deficiency are quite complex. Nevertheless, some of the changes are typical and can be explained by the functions of Zn in specific enzyme reactions or steps in particular metabolic pathways. By affecting expression and regulation of genes and defence mechanisms, Zn contributes to plant tolerance to environmental stress factors.
4.1. 10.4.1 Protein Synthesis

In Zn-deficient plants, the rate of protein synthesis and the protein concentration are strongly reduced, whereas amino acids accumulate. Upon resupply of Zn to deficient plants, protein synthesis resumes quite rapidly. Besides the functions of Zn described above, at least two other functions of Zn in protein metabolism are responsible for these changes. Zinc is a structural component of ribosomes and essential for their structural integrity. The Zn concentration of ribosomal RNA in Zn-sufficient cells of Euglena is in the range of 650 to 1280 μg g⁻¹ RNA, whereas in Zn deficient cells it is 300 to 380 μg g⁻¹ RNA. In the absence of Zn, ribosomes disintegrate, but can be reconstituted after resumption of Zn supply.

In shoot meristems of rice, disintegration of the 80S ribosomes (soluble fraction in the cytoplasm) takes place when the Zn concentration is below 100 μg g⁻¹ dw. Considerably lower Zn concentrations are required to decrease protein concentration. In tobacco tissue culture cells, the corresponding concentrations were 70 μg Zn for a decrease in 80S ribosomes and 50 μg Zn for a decrease in protein concentration.

A particularly high Zn requirement for protein synthesis has been also shown in pollen tubes, where the Zn concentration at the growing tip was about 150 μg g⁻¹ dw compared with about 50 μg g⁻¹ in more basal regions. In the newly emerged root tips of wheat plants, Zn concentrations are about 220 μg g⁻¹. In the shoot meristems, and presumably also in other meristematic tissues, a Zn concentration of at least 100 μg g⁻¹ dw is required for maintenance of protein synthesis. This is about 5–10 times more than the adequate Zn concentration in mature leaf blades. For other nutrients, this gradient is usually less steep. To meet the high Zn demand in the shoot meristem, most of the root-supplied Zn is preferentially translocated to the shoot meristem, via xylem–phloem transfer in the stem.

4.2. 10.4.2 Tryptophan and Indoleacetic Acid Synthesis

The most distinct Zn deficiency symptoms – stunted growth and ‘little leaf’ – are presumably related to disturbance in the metabolism of auxins, indoleacetic acid (IAA) in particular. The mode of action of Zn in auxin metabolism is still unclear. In Zn-deficient tomato plants, retarded stem elongation is correlated with a decrease in IAA concentration; upon resupply of Zn, stem elongation and IAA concentrations increase. The response to the Zn treatment was more rapid for IAA concentrations than for elongation growth. Low concentrations of IAA in Zn-deficient plants may be the result of inhibited synthesis or enhanced degradation of IAA. Tryptophan is most likely the precursor for the biosynthesis of IAA (Fig. 39.):

10.3. ábra - Figure 39. Biosynthesis of IAA

In leaves of Zn-deficient plants, tryptophan concentrations increase similarly to other amino acids, most likely as a result of impaired protein synthesis. Although the lower IAA concentration in Zn-deficient leaves may indicate a role for Zn in the biosynthesis of IAA from tryptophan, lower IAA concentrations are more likely the result of enhanced oxidative degradation of IAA. Adequate Zn nutrition also increases the concentrations of endogenous gibberellins. The low concentrations of IAA and gibberellins may be the cause for the stunted growth and ‘little leaf’ formation under Zn deficiency.

4.3. 10.4.3 P-Zn Interactions

High application rates of P fertilizers to soils low in available Zn can induce Zn deficiency, by altering either soil or plant factors. In soil, high P concentrations can decrease solubility of Zn. However, this is not always the case. High P supply is often associated with a reduction in root growth and a lesser degree of colonization of
roots with arbuscular mycorrhiza (AM). Both these factors are important for the acquisition of Zn. In wheat, P fertilization reduced grain Zn concentration by 33 to 39 % and root colonization with AM by 33 to 75 %. The decrease in grain Zn concentration by P fertilization was also related to dilution of Zn due to increased grain yield with P fertilization. Similarly, the decrease in Zn concentration in shoots and an induction of Zn deficiency symptoms by high P supply is the result of enhanced shoot growth and, thus, ‘dilution’ of Zn in the plants. There are, however, additional physiological interactions between P and Zn within the plants involved. With increasing P concentration in the shoot, Zn deficiency symptoms become more severe, although the Zn concentration is not decreased. However, the physiological availability of Zn is decreased as indicated, for example, in lower proportions of water extractable Zn and lower SOD activity in leaves. P concentration in the shoot may therefore decrease solubility and mobility of Zn both within the cells and in long-distance transport to the shoot apex.

In solution culture at high P but low Zn supply, the P-induced Zn deficiency is often associated with very high P concentrations and symptoms of P toxicity in mature leaves, which may be mistaken for evidence of accentuation of Zn deficiency because of the large P/Zn ratio. Zn uptake is not affected by increasing P concentrations in the external solution. In the absence of Zn, or with low external concentrations, however, the P concentration in the shoot is very high, leading to toxicity symptoms. In general, a P concentration greater than 20 mg kg⁻¹ leaves can be considered as toxic.

The main reason for the high P concentration in the leaves is that Zn deficiency enhances the P uptake rate by the roots and its translocation to the shoots. Zinc deficiency also increases the permeability of the plasma membrane of root cells to P, as well as to Cl and B, and may even lead to B toxicity.

Thus, enhanced P uptake in Zn-deficient plants can in part be due to higher passive permeability of the plasma membranes of root cells or impaired control of xylem loading.

4.4. 10.4.4 Zn binding Forms and Bioavailability

Much is known about the localization and binding forms of Zn in seeds and grains; however less is known for vegetative organs. In grains and seeds, most of the Zn and other nutrients are localized in so-called ‘protein bodies’ in the form of discrete particles, the globoïd crystals. These globoïds mainly consist of phytate, i.e. salts of phytic acid. In wheat seeds, similarly high Zn concentrations (600 μg g⁻¹ dw) were found in the scutellum. Zinc, Fe and proteins are generally co-localized within seed tissues and there is a very high positive correlation between the concentrations of Zn, Fe and protein in seeds of a number of germplasms. These results suggest that protein is a sink for Zn and Fe. A recent speciation analysis in the barley embryo fraction showed that Fe is bound to phytic acid whereas Zn is mainly associated with proteins or peptides.

Phytic acid is a strong negatively charged compound and has high affinity to bind divalent cations such as Zn, forming insoluble or unavailable Zn-phytate complexes in seeds. The strong binding of Zn to phytic acid is of concern to nutritionists as it reduces the bioavailability of Zn for monogastric animals and man. A negative correlation occurs, for example, in soybean products between phytic acid (phytate) concentration and the function of nutrients in plant physiological processes, deficiency symptoms II.

Thus, enhanced P uptake in Zn-deficient plants can in part be due to higher passive permeability of the plasma membranes of root cells or impaired control of xylem loading.

4.5. 10.4.5 Zn Deficiency

Zinc deficiency is widespread among plants grown in highly weathered acid soils and in calcareous soils. In the latter case, Zn deficiency is often associated with Fe deficiency (‘lime chlorosis’). The low availability of Zn in calcareous soils of high pH is mainly due to the adsorption of Zn to clay or CaCO₃, rather than from the formation of sparingly soluble Zn(OH)₂ or ZnCO₃. In addition, Zn uptake and translocation to the shoot are inhibited by high concentrations of bicarbonate, HCO₃⁻. This effect is very similar to the effect of HCO₃⁻ on Fe. In contrast to Fe deficiency, however, Zn deficiency in plants grown in calcareous soils can be corrected quite readily by application of inorganic Zn salts such as ZnSO₄ to the soil.

The most characteristic visible symptoms of Zn deficiency in dicotyledonous plants are stunted growth due to shortening of internodes (‘rosetting’) and a drastic decrease in leaf size (‘little leaf’). Under severe Zn
deficiency, the shoot apices die (‘die-back’) as, for example, in forest plantations in South Australia. Quite often these symptoms are combined with chlorosis, which is either highly contrasting or diffusive (‘mottle leaf’). These symptoms are usually more severe at high light intensity than in partial shade. Similarly, plants are more susceptible to low Zn supply when exposed to heat and drought stress. In cereals such as wheat, typical symptoms are reduction in shoot elongation and development of whitish-brown necrotic patches on middle-aged leaves, whereas young leaves remain yellowish green in colour, but show no necrotic lesions. Symptoms of chlorosis and necrosis in older leaves of Zn-deficient plants are often secondary effects caused by P or B toxicity, or by photooxidation resulting from impaired export of photosynthates.

Under Zn deficiency, shoot growth is usually more inhibited than root growth, and root growth may even be enhanced at the expense of the shoot growth.

4.6. 10.4.6 Zn Toxicity

Zinc toxicity is observed very rarely in crop plants and occurs mainly in soils contaminated by mining and smelting activities and treated with sewage sludge. At very high Zn supply, Zn toxicity can readily be induced in non-tolerant plants with inhibition of root elongation being a very sensitive parameter. Quite often, Zn toxicity leads to chlorosis in young leaves. This may be an induced deficiency of, for example, Mg or Fe, because of the similar ion radius of Zn2+ and Fe2+ and Zn2+ and Mg2+. Induced Mn deficiency may also be of importance, as high Zn supply strongly decreases the Mn concentration of plants.

The critical toxicity concentrations in leaves of crop plants range from 100 μg Zn g⁻1 dw to more than 300 μg Zn g⁻1, the latter values being more typical. Increasing soil pH by liming is the most effective strategy for decreasing Zn concentration and zinc toxicity in plants. In comparison with the genotypical differences between wild plants, differences in zinc tolerance between crop plants are small, but marked, even within the same species.

5. 10.5 Molybdenum

Molybdenum is a transition element; it is present in small amounts in the lithosphere (average 2.4 mg kg⁻1) and in soils (ranging from 0.2 to 36 mg kg⁻1). In aqueous solution with a pH >4.3, Mo occurs mainly as the molybdate oxyanion, MoO₄²⁻, in its highest oxidized form (Mo(VI)). At lower pH (<4.3), protonated species (HMoO₄⁻, MoO₃(H₂O)₃) become the prevailing forms. At high concentrations (>10⁻⁴ M) and low pH, molybdate can polymerize; but this is unlikely to occur in soil solution because soluble Mo is usually <10⁻⁶ M. Due to its electron configuration, Mo(VI) shares many chemical similarities with vanadium (V) and, particularly, tungsten (W). In fact, many anaerobic archaea and some bacteria require tungsten, but not Mo. Several properties of the molybdate anion MoO₄²⁻ also resemble those of the divalent inorganic anion sulphate (SO₄²⁻), which has important implications for Mo availability in soils and uptake by plants. In long-distance transport in plants, Mo is readily mobile in xylem and phloem. The form in which Mo is translocated is unknown, but its chemical properties indicate that it is most likely transported as MoO₄²⁻ rather than in complexed form.

The requirement of plants for Mo is lower than that for any of the other nutrients. The functions of Mo as a plant nutrient are related to the valency changes it undergoes as a metal component of enzymes. Within these enzymes, Mo shuttles between three oxidation states (+4, +5 and +6), thereby catalysing two-electron transfer reactions. In higher plants, only few enzymes have been found to contain Mo as a cofactor, including nitrate reductase, xanthine dehydrogenase, aldehyde oxidase and sulphite reductase. In addition, Mo is a cofactor of nitrogenase in N₂-fixing bacteria. The functions of Mo are therefore closely related to N metabolism, and the Mo requirement strongly depends on the mode of N supply.

5.1. 10.5.1 Mo Uptake

A molybdate-specific transporter has been identified in Arabidopsis thaliana. This transporter, MOT1, has a high affinity for MoO₄²⁻ with a Km of 20 nM in an uptake assay with yeast expressing the MOT1 gene. MOT1 belongs to the sulphate transporter superfamily, but does not appear to mediate sulphate transport. It is expressed in both roots and leaves and the protein appears to be localized in the mitochondria. Mutants lacking MOT1 had markedly decreased Mo concentrations in roots and shoots. Natural variation in Mo accumulation among different accessions of Arabidopsis thaliana is, to a large extent, related to the expression level of MOT.
In addition to MOT1, it is likely that some non-specific transporters also contribute to Mo uptake by plants, particularly sulphate transporters. For example, the high-affinity sulphate transporter from the tropical legume Stylosanthes hamata, SHST1, is able to mediate MoO$_4^{2-}$ uptake into yeast cells expressing the SHST1 gene from the external medium containing nM concentrations of molybdate. There are also numerous reports in the literature showing that molybdate uptake is suppressed by sulphate. The effect of sulphate can be two-fold: a direct competition for the transporters and regulation of the expression of sulphate transporter genes by plant S status. In field-grown wheat, S deficiency resulted in greatly increased transcript abundance of sulphate transporters such as Sultr1;1 and Sultr4;1, but not Sultr5;2 which is the wheat homologue of the Arabidopsis MOT1. Molybdenum concentrations in leaves and ears of S-deficient wheat were about double of those in S-sufficient plants.

5.2. 10.5.2 Nitrogenase

Nitrogenase is the key enzyme complex unique to all N$_2$-fixing microorganisms. It consists of two Fe proteins, one of which is the FeMo protein containing two unique metal centres, the P-cluster (8Fe-7S) and the FeMo cofactor.

In some free-living diazotrophic bacteria (e.g., Azotobacter chroococcum) in addition to the Mo-nitrogenase, another nitrogenase occurs in which Mo is replaced by vanadium.

Legumes and non-legumes dependent on N$_2$fixation have a high Mo requirement, particularly in root nodules. When the external supply is low, the Mo concentration of the nodules is usually higher than that of leaves, whereas when the external supply is high, the concentration in the leaves increases more strongly than in the nodules. When Mo is limiting, preferential accumulation in root nodules may lead to a considerably lower Mo concentration in the shoot and seeds of nodulated legumes. However, the relative allocation of Mo to the various plant organs varies considerably not only between plant species, but also between genotypes within a species, for example in Phaseolus vulgaris.

As would be expected, the growth of plants relying on N$_2$fixation is particularly stimulated by the application of Mo to deficient soils and nodule dry weight can increase 18-fold which indirectly reflects the increase in the capacity for N$_2$fixation by improved Mo supply.

In soils low in Mo availability, the effect of application of Mo to legumes depends on the form of N supply. Mo applied to nodulating and non-nodulating soybean plants increased N concentration and seed yield only in the nodulated plants without or with insufficient supply of N fertilizer. This demonstrates the greater requirement for molybdenum in N$_2$fixation than in nitrate reduction. It also indicates that on soils with low Mo availability, it is possible to replace the application of N fertilizer to legumes by application of Mo fertilizer combined with rhizobium inoculation.

Low availability of Mo in tropical forest soil may limit N$_2$fixation by free-living heterotrophic bacteria, thus impacting on N cycling. Mo addition to weathered tropical forest soils from Panama significantly increased N$_2$ fixation.

5.3. 10.5.3 Mo Deficiency and Toxicity

Depending on plant species and N source, the critical deficiency levels of Mo vary between 0.1 and 1.0 μg g$^{-1}$ leaf dw. In seeds the Mo concentration is highly variable but, in general, much higher in legumes than in non-legumes. Molybdenum is unique among the essential elements in that normal seeds of some plants may store more Mo than required by the next generation plant.

In Mo-deficient plants, symptoms of N deficiency and stunted growth and chlorosis in young leaves are common. In dicotyledonous species, a strong reduction in size and irregularities in leaf blade formation (whiptail) are the most typical visual symptoms, caused by local necrosis in the tissue and insufficient differentiation of vascular bundles in the early stages of leaf development.

Local chlorosis and necrosis along the main veins of mature leaves (e.g., ‘Yellow spot’ in citrus) and whiptail in young leaves may reflect the same type of local metabolic disturbances, occurring however, at different stages of leaf development. When there is severe deficiency, marginal chlorosis and necrosis on mature leaves with a high nitrate concentration also occur.
Molybdenum deficiency is widespread in legumes and certain other plant species (e.g., cauliflower and maize) grown in acid mineral soils with large concentrations of reactive Fe oxidihydrate and thus a high capacity for adsorbing MoO$_4^{2-}$. Furthermore, adsorption of molybdate increases with decreasing soil pH. The effect of the liming treatment alone on the plant dry weight is similar to the application of Mo to the unlimed soil. Thus, quite often liming and Mo application can be seen as alternatives for stimulating legume growth on acid mineral soils. Responses of legume growth to liming therefore also strongly depend on the Mo availability in the soils. A combination of both liming and Mo supply often leads to luxury uptake and very high Mo concentrations in the vegetative parts of the shoots and seeds. A high Mo concentration in seeds ensures proper seedling growth and high final grain yields in plants growing in soils low in available Mo. Hence, the effect of Mo application to a deficient soil on plant growth is negatively related to the seed Mo concentration and the amount of Mo applied to the seed crop.

Compared with the uptake rates of other micronutrients, the rate of Mo uptake by soybean plants during the first 4 weeks after germination is very low; thus the Mo requirement for growth has to be met mainly by retranslocation from the seed. Large-seeded cultivars combined with high Mo availability during the seed-filling period are therefore very effective in the production of seeds suitable for soils low in available Mo.

As Mo is highly phloem-mobile, foliar application is an appropriate and easy procedure for correcting acute Mo deficiency. In legumes, Mo applied as a foliar spray in the early growth stages is preferentially translocated into the nodules and very effective in increasing final yield, for example, in soybean or groundnut. Compared with soil application, foliar application to groundnut not only increases yield but also N uptake and the Mo concentration in the shoots, seeds and nodules. Foliar sprays of Mo applied before flowering are effective in correcting Mo deficiency in grapevine.

A lower effectivity of soil compared with foliar applied Mo may reflect fixation of Mo in the soil; however, it is often also the result of impaired uptake by the roots. Sulphate and molybdate are strongly competing anions during uptake by the roots. Therefore, sulphate-containing soil amendments such as gypsum, as well as single superphosphate (SSP, which contains sulphate), reduce Mo uptake.

The reduction in Mo uptake by sulphate may also be of significance for natural ecosystems. In red cedar trees, there is a negative relationship between Mo and S concentration of tree rings, the increase in S concentration being closely related to the historical trend in coal production and, thus, SO$_2$ emission in the area in which the trees were growing.

A unique feature of Mo nutrition is the wide variation between the critical deficiency and toxicity concentrations which may differ by a factor of up to 104 (e.g., 0.1–1,000 μg Mo g$^{-1}$ dw) as compared with a factor of 10 or less for B or Mn. Plants are generally quite tolerant to Mo toxicity. Under Mo toxicity, malformation of the leaves and a golden yellow discoloration of the shoot tissue occur, most likely due to the formation of molybdocatechol complexes in the vacuoles. In oilseed rape and tomato, the most striking symptoms of Mo toxicity is a dark blue coloration of stems, which is due to the formation of molybdenum–anthocyanin complexes. Genotypic differences in tolerance to Mo toxicity are closely related to differences in the translocation of Mo from roots to shoots.

High, but non-toxic, concentrations of Mo in plants are advantageous for seed production, but such concentrations in forage plants may be dangerous for animals, and for ruminants in particular, which are very sensitive to excessive concentrations of Mo. Molybdenum concentrations above 5 to 10 mg kg$^{-1}$ dw of forage can induce toxicity known as molybdenosis (or ‘teart’). This occurs, for example, in western parts of the United States, Australia and New Zealand, often in soils with poor drainage and high in organic matter content, or on pastures established on retorted oil shale disposal piles. Molybdenosis is caused by an imbalance of Mo and Cu in the ruminant diet, i.e. an induced Cu deficiency. The depressing effect of sulphate on molybdate uptake can be used to reduce the Mo concentrations in plants to non-toxic levels either for the plants themselves or for the ruminants.

Molybdenum nutrition of plants growing in mixed pastures of legumes, herbs and grasses therefore requires special consideration. On the one hand, the relatively large requirement of legumes for N$_2$ fixation and for Mo in the seeds must be met, but at the same time toxic concentrations in the forage of grazing animals must be avoided.

6. 10.6 Literature
10. The function of nutrients in plant physiological processes, deficiency symptoms II.


10. The function of nutrients in plant physiological processes, deficiency symptoms II.


7. 10.7 Questions

1. What is the role of siderophores in the Fe-uptake?

2. Deficiencies of iron, magnesium, and nitrogen all cause chlorosis. Iron chlorosis develops only between the veins of young leaves while chlorosis due to both magnesium and nitrogen deficiencies develops more generally in older leaves. Explain these differences. Why does each deficiency lead to chlorosis and why are the patterns different?

3. What are the main symptoms of zinc deficiency?
11. fejezet - 11. Nitrogen metabolism in plants

1. 11.1 Nitrogen

After carbon, nitrogen (N) is the element required in largest amounts by plants: about 1–5% of total plant dry matter consists of N, which is an integral constituent of proteins, nucleic acids, chlorophyll, co-enzymes, phytohormones and secondary metabolites. The availability of N to roots is therefore a decisive factor for plant growth. Atmospheric N2 is only available to plants that are capable of forming symbiosis with N2-fixing soil bacteria. Most plants therefore depend on other N compounds for their growth. The major sources of N taken up by the roots of higher plants are nitrate (NO3-) and ammonium (NH4+). In order to increase crop production, approximately 100 million tons of N fertilizers were applied globally in 2008. A large proportion, approx. 60%, of the total N fertilizer used is for cereal crops. Generally, only 40–50% of the applied N fertilizer is utilized by the crop. The N which is lost from the plant–soil system can result in environmental problems, including water and air pollution.

This chapter describes N acquisition by roots, N assimilation, functions of N compounds and the effect of N on plant growth and composition. Classical physiological observations of N responses are linked with the underlying molecular mechanisms that have recently been unravelled in order to provide integrated insight into the principles of N nutrition of higher plants.

Nitrate and ammonium are the major sources of inorganic N taken up by the roots of higher plants. Nitrate is generally present in higher concentrations (1–5 mM) than ammonium (20–200 μM) in the soil solution of agricultural soils. Nitrate is also more mobile in the soil than ammonium and therefore more available to plants.

In unfertilized agricultural soils, ammonium can be present in higher concentrations than nitrate, and amino acids provide an additional source of N. Amino acid concentrations in the soil solution range between 0.1 and 100 μM and they dominate the pool of N bound to soil particles.

Ammonium and amino acids are also the dominating plant-available N forms in acid forest soils. Due to limited nitrification in anaerobic soils, rice paddy soils also contain more ammonium than nitrate.

2. 11.2 The nitrogen cycle

The global nitrogen supply is generally distributed between three major pools: the atmospheric pool, the soil (and associated groundwater) pool, and nitrogen contained within the biomass. Central to the idea of a nitrogen cycle (Fig.40) is the pool of nitrogen found in the soil. Nitrogen from the soil pool enters the biomass principally in the form of nitrate (NO3-) taken up by plants and microorganisms. Once assimilated, nitrate nitrogen is converted to organic nitrogen in the form of amino acids and other nitrogenous building blocks of proteins and other macromolecules. Nitrogen moves further up the food chain when animals consume plants. Nitrogen is returned to the soil through animal wastes or the death and subsequent decomposition of all organisms.

11.1. ábra - Figure 40. The nitrogen cycle, illustrating relationships between the three principal nitrogen pools: atmospheric, soil, and biomass
3.11.3 Ammonification, nitrification and denitrification are essential process in the nitrogen cycle

In the process of decomposition, organic nitrogen is converted to ammonia by a variety of microorganisms including the fungi. This process is known as ammonification (Fig.41). Some of the ammonia may volatilize and reenter the atmosphere, but most of it is recycled to nitrate by soil bacteria. The first step in the formation of nitrate is the oxidization of ammonia to nitrite (NO2−) by bacteria of the genera Nitrosomonas or Nitrococcus. Nitrite is further oxidized to nitrate by members of the genus Nitrobacter. These two groups are known as nitrifying bacteria and the result of their activities is called nitrification. Nitrifying bacteria are chemoheterotrophs; that is, the energy obtained by oxidizing inorganic substances such as ammonium or nitrite is used to convert carbon dioxide to organic carbon. In taking up nitrate from the soil, plants must compete with bacteria known as denitrifiers (e.g., Thiobacillus denitrificans). By the process of denitrification, these bacteria reduce nitrate to dinitrogen, which is then returned to the atmosphere. Estimates for the amount of nitrogen lost to the atmosphere by denitrification range from 93 million to 190 million metric tons annually.
4. 11.4 Nitrogen Assimilation

Nitrate (NO$_3^-$) is readily mobile in the xylem and can also be stored in the vacuoles of roots, shoots and storage organs. In order for the N in nitrate to be incorporated into organic structures, nitrate has to be reduced to ammonium (NH$_4^+$). Most of the ammonium, whether originating from nitrate reduction or from direct uptake from the soil solution, is normally incorporated into organic compounds in the roots, although some NH$_4^+$ may also be translocated to the shoot even in plants receiving nitrate as the sole N form. The importance of reduction and assimilation of nitrate for the life of plants is similar to that of the reduction and assimilation of CO$_2$ in photosynthesis. Nitrogen assimilation is intricately regulated. This is necessary in order to integrate environmental signals with carbon metabolism so that N assimilation is coupled with the availability of N in the soil and the demand for synthesis of various N-containing compounds as well as with the availability of C skeletons, energy and reductants for the assimilatory pathway.

5. 11.5 Plants generally take up nitrogen in the form of nitrate

Except in extreme situations noted earlier, nitrate (NO$_3^-$) is the more abundant form of nitrogen in soils and is most available to plants that do not form nitrogen-fixing associations. However, in spite of numerous studies describing the physiology of NO$_3^-$ uptake, there is a great amount of uncertainty surrounding the mechanism of NO$_3^-$ transport into roots. It has been shown in various studies that uptake of NO$_3^-$ is sensitive to (1) low temperature, (2) inhibitors of both respiration and protein synthesis, and (3) anaerobic conditions. All of these results support the hypothesis that NO$_3^-$ transport across the root cell membrane is an energy-dependent process mediated by a carrier protein (Fig. 42.).

11.3. ábra - Figure 42. N-cycle in plants
In root cells that have never been exposed to nitrate, there appears to be a limited capacity for NO3- uptake. This suggests a small amount of carrier is present in the membrane at all times (that is, a constitutive protein). On exposure to external nitrate, the rate of uptake increases from two- to fivefold, but addition of inhibitors of protein synthesis causes the rate to fall rapidly back to the constitutive level. This pronounced sensitivity of NO3- uptake to inhibitors of protein synthesis suggests that the bulk of the carrier protein is inducible, that is, the presence of NO3- in the soil stimulates the synthesis of new carrier protein. Once inside the root, NO3-
may be stored in the vacuole, assimilated in the root cells, or translocated in the xylem to the leaves for assimilation. Nitrate cannot be assimilated directly but must first be reduced to NH4+ in order to be assimilated into organic compounds. This is a two-step process, the first being the reduction of NO3- to nitrite (NO2-) by the enzyme nitrate reductase (NR). NR is generally assumed to be a cytosolic enzyme.

\[ 2H^+ + NO_3^- + 2e^- \rightarrow NO_2^- + H_2O \]

The product NO2- then moves into plastids (in roots) or chloroplasts (in leaves) where it is quickly reduced to NH4+ by the enzyme nitrite reductase (NiR). In leaves, the electrons required for the reduction of NO2- to NH4+ are generated

\[ 8H^+ + NO_2^- + 6e^- \rightarrow NH_4^+ + 2H_2O \]

by photosynthetic electron transport. Thus, the assimilation of NO3- competes with the assimilation of CO2 for photosynthetic electrons. Consequently, NO3- assimilation in leaves can also be considered a photosynthetic process similar to CO2 assimilation. The interactions between carbon and nitrogen metabolism indicate the importance of photosynthetic electron transport in overall primary reductive metabolism.

### 6. 11.6 Nitrate Reduction

The reduction of nitrate to ammonium is mediated by two enzymes: nitrate reductase, which catalyses the two-electron reduction of nitrate to nitrite (NO2-), and nitrite reductase, which transforms nitrite to ammonium in a six-electron transfer process (Fig. 43).

Nitrate reductase (NR) is a cytosolic enzyme consisting of two identical subunits, each with three co-factors covalently bound to specific domains of the enzyme. The three co-factors which participate in the transfer of electrons from NADH/NADPH to nitrate are flavine adenine dinucleotide (FAD), a heme (bound to a domain which resembles a family of cytochromes) and molybdopterin (a molybdenum containing co-factor). Most plant species have two nitrate reductase (NIA) genes which are expressed in shoots and roots.

**11.4. ábra - Figure 43.** The reduction of nitrate to ammonia is a multistep reaction in which nitrates are reduced to nitrites, which are then converted to hyponitrites then to hydroxylamines and finally to ammonia
The nitrite generated by nitrate reductase is transported to the chloroplast for reduction to ammonium by nitrite reductase. Nitrite reductase is encoded by a single gene in higher plants. It is localized in the chloroplasts in leaves and in the proplastids of roots and other non-green tissues. In green leaves, the electron donor is reduced ferredoxin, generated by photosystem I during photosynthetic electron transport in the light. Electrons from the reduced ferredoxin are passed to nitrite via a ferredoxin-binding domain, an iron–sulphur cluster, and a siroheme co-factor bound to the nitrite reductase enzyme. In the root plastids, reduced ferredoxin is generated via NADPH in the pentose phosphate pathway coupled with ferredoxin-NADP reductase.

To prevent accumulation of nitrite, which is toxic to plant cells, nitrate reductase activity is regulated by several mechanisms. The regulation is exerted at different levels, including enzyme synthesis, degradation and reversible inactivation as well as regulation of effectors and the concentration of substrate. The enzyme has a half-life of only a few hours and is absent in plants not receiving nitrate. The expression of the nitrate reductase genes is strongly and rapidly induced by nitrate, leading to active protein within a few hours following addition of nitrate. Additionally, the concentration of nitrate reductase protein is increased by light, sucrose and cytokinin, whereas glutamine, a primary product of N assimilation, represses nitrate reductase. This regulation links the capacity for nitrate assimilation with the availability of sugars to provide C skeletons. Elevated atmospheric carbon dioxide can reduce the assimilation of nitrate because the reductants produced by photosynthesis are necessary for both carbon and nitrate assimilation.

Nitrate reductase is further regulated by several post-translational mechanisms. A protein kinase phosphorylates nitrate reductase and thereby enables binding of a protein which inactivates the enzyme. The inactivation of nitrate reductase by protein binding is inhibited by triose and hexose phosphates. This ensures that nitrate reductase is maintained in an active state when there is ample supply of C skeletons for amino acid synthesis. Also, enzyme activity can be restored by dephosphorylation by a phosphatase which prevents protein binding and inhibition. During short-term light–dark transitions, post-translational inhibition of nitrate reductase occurs within a few minutes, preventing accumulation of nitrite.

The close correlation between light intensity and nitrate reduction in green leaves may reflect fluctuations in carbohydrate concentrations and in the corresponding supply of reducing equivalents and C skeletons. The diurnal fluctuations in nitrate reductase activity may lead to a decrease in the foliar nitrate concentrations during the light period. Plants grown permanently under low-light conditions (e.g., in glasshouses during winter) may contain nitrate concentrations which are several fold higher than those of plants grown under high-light conditions (e.g., in an open field during the summer). This is particularly evident in certain vegetables belonging to the Brassicaceae or Chenopodiaceae; for example, spinach has a high preference for nitrate accumulation in the shoots and uses nitrate accumulation in vacuoles for osmoregulation. Under low-light conditions, nitrate concentrations in spinach leaves can reach 100 mM nitrate, corresponding to 6,000 mg kg⁻¹ fresh weight.

7. 11.7 Ammonium Assimilation

Ammonium is a central intermediate in plant N metabolism. Besides uptake from the soil by roots, ammonium is constantly generated in high rates in plant tissues by processes such as nitrate reduction, photorespiration, lignin biosynthesis, senescence-induced N remobilization and N2 fixation in legumes. Irrespective of the source of ammonium or the organ in which it is assimilated (roots, root nodules and leaves) the key enzymes involved
are glutamine synthetase (GS) and glutamate synthase (GOGAT; glutamine-oxoglutarate aminotransferase). Both enzymes are present in roots, in chloroplasts and in N2-fixing microorganisms. Assimilation of most, if not all, ammonium derived from ammonium uptake, N2fixation, nitrate reduction and photorespiration is mediated by the glutamine synthetase–glutamate synthase pathway. In this pathway the amino acid glutamate acts as the acceptor for ammonium, forming the amide glutamine.

Glutamine synthetase exists in multiple enzyme forms located in the cytosol and in plastids. Cytosolic GS has multiple metabolic functions such as assimilation of ammonium into glutamine for transport and distribution throughout the plant. During leaf senescence cytosolic GS fulfils a key function in the assimilation and recycling of ammonium generated from various catabolic processes. This role is particularly important after anthesis and during grain development and filling in cereals, when N is remobilized to the reproductive sinks. Several of the isoenzymes of the cytosolic GS1 gene family are abundantly expressed in roots and can be classified into high-affinity or low-affinity subtypes differing in Vmax values. Some are more abundant under N deficiency while others dominate under high external ammonium supply. This dynamic regulation may contribute to the homeostatic control of glutamine synthesis in roots.

8. 11.8 Ammonium Toxicity

Plant species differ in tolerance to ammonium. Among crop plants, barley is ammonium-sensitive, whereas rice is ammonium-tolerant. The symptoms of ammonium toxicity include leaf chlorosis, stunted growth and eventually necrotic leaves and plant death.

Various hypotheses have been put forward to explain the physiological processes underlying ammonium toxicity. When whole tissue of ammonium-fed plants is analyzed, several chemical changes are observed. Generally, compared to nitrate-fed plants, there is an accumulation of ammonium ions, inorganic anions such as chloride, sulphate and phosphate as well as of amino acids. In contrast, there is a reduction in the concentration of the essential cations such as K+, Ca2+and Mg2+ as well as organic acids such as malate. These and other observations have led to the hypotheses that ammonium toxicity may be the result of (i) decreased uptake of essential cations, (ii) ammonium-induced disorders in pH regulation, or (iii) excessive consumption of sugars for ammonium assimilation causing carbohydrate limitation.

Ammonium influx into the roots of the ammonium-sensitive species barley appears to be much higher than into the ammonium-tolerant species rice, suggesting that rice can control the influx of ammonium into the roots. barley on the other hand, releases ammonium back into the soil. This has led to the hypothesis that in ammonium-sensitive species, the apparently futile transmembrane cycling of ammonium and the operation of an energy-intensive ammonium efflux mechanism may be the cause of ammonium toxicity.

Additionally, the acidification of the rhizosphere induced by ammonium uptake may in itself pose a stress to plants, particularly in acid soils where it can increase Al toxicity.

Each of these factors may contribute to plant ammonium toxicity depending on the plant species and particular growth conditions.

9. 11.9 Some nitrogen-fixing bacteria are free-living organisms

Free-living, nitrogen-fixing bacteria are widespread. Their habitats include marine and freshwater sediments, soils, leaf, and bark surfaces, and the intestinal tracts of various animals. Although some species are aerobic (e.g., Azotobacter, Beijerinckia), most will fix dinitrogen only under anaerobic conditions or in the presence of very-low-oxygen partial pressures (a condition known as microaerobic). These include both nonphotosynthetic genera (Clostridium, Bacillus, Klebsiella) and photosynthetic genera (Chromatium, Rhodospirillum) of bacteria. In addition to the bacteria, several genera of cyanobacteria (principally Anabaena, Nostoc, Lyngbia, and Calothrix) are represented by nitrogen-fixing species. Although free-living nitrogen-fixing organisms are widespread, most grow slowly and, except for the photosynthetic species, tend to be confined to habitats rich in organic carbon. Because a high proportion of their respiratory energy is required to fix dinitrogen, less energy is therefore available for growth.
10. 11.10 Symbiotic nitrogen fixation involves specific associations between bacteria and plants

Several types of symbiotic nitrogen-fixing associations are known, including the well-known association between various species of bacteria and leguminous plants. Some of the more important associations are listed in Table 2.


<table>
<thead>
<tr>
<th>Bacterium</th>
<th>Host plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azorhizobium</td>
<td>Sesbania</td>
</tr>
<tr>
<td>Bradyrhizobium japonicum</td>
<td>Glycine(soybean)</td>
</tr>
<tr>
<td>Rhizobium meliloti</td>
<td>Medicago(alfalfa), Melilotus(sweet clover)</td>
</tr>
<tr>
<td>Rhizobium leguminosarum</td>
<td>Lathyrus(sweet pea), Trifolium(clover)</td>
</tr>
<tr>
<td>biovar viciae</td>
<td>Lens(lentil), Pisum(garden pea), Vicia (vetch, broad bean)</td>
</tr>
<tr>
<td>biovar trifolii</td>
<td>Trifolium(clover)</td>
</tr>
<tr>
<td>biovar phaseoli</td>
<td>Phaseolus(bean)</td>
</tr>
<tr>
<td>Rhizobium loti</td>
<td>Lotus(bird’s-foot trefoil)</td>
</tr>
</tbody>
</table>

In symbiotic associations the plant is identified as the host and the microbial partner is known as the microsymbiont. The most common form of symbiotic association results in the formation of enlarged, multicellular structures (Fig.44.), called nodules, on the root (or occasionally the stem) of the host plant.

11.5. ábra - Figure 44. Nitrogen-fixing nodules on roots of soybean (Glycine max)

In the case of legumes, the microsymbiont is a bacterium of one of three genera: Rhizobium, Bradyrhizobium, or Azorhizobium. Collectively, these organisms are referred to as rhizobia. Curiously, only one nonleguminous genus, Parasponia (of the family Ulmaceae), is known to form root nodules with a rhizobia symbiont. The rhizobia are further divided into species and subgroups called biovars (a biological variety) according to their host range. Most rhizobia are restricted to nodulation with a limited number of host plants while others are highly specific, infecting only one host species. Nodules are also found in certain nonleguminous plants such as alder (Alnus), bayberry (Myrica), Australian pine (Casuarina), some members of the family Rosaceae, and certain tropical grasses. However, the microsymbiont in these nonleguminous nodules is a filamentous bacterium (Frankia) of the group...
actinomycetes. Both Rhizobium and Frankia live freely in the soil but fix dinitrogen only when in symbiotic association with an appropriate host plant. A limited number of non-nodule-forming associations have been studied, such as that between Azolla and the cyanobacterium Anabaena. Azolla is a small aquatic fern that harbors Anabaena in pockets within its leaves. In southeast Asia, Azolla has proven useful as green manure in the rice paddy fields where it is either applied as a manure or co-cultivated along with the rice plants. Because more than 75 percent of the rice acreage consists of flooded fields, free-living cyanobacteria and anaerobic bacteria may also make a significant contribution. These practices have allowed Asian rice farmers to maintain high productivity for centuries without resorting to added chemical fertilizers.

11. 11.11 Rhizobia infect the host roots, which induces nodule development

The sequence of events beginning with bacterial infection of the root and ending with formation of mature, nitrogen-fixing nodules has been studied extensively in the legumes, historically from the morphological perspective and more recently from the biochemical/molecular genetic perspective. Overall, the process involves a sequence of multiple interactions between the bacteria and the host roots. In effect, the rhizobia and the roots of the prospective host plant establish a dialogue in the form of chemical messages passed between the two partners. Based on studies carried out primarily with Glycine, Trifolium, and Pisum, as many as nine or ten separate developmental stages have been recognized. In order to simplify our discussion, however, we will consider the events in four principal stages:

1. Multiplication of the rhizobia, colonization of the rhizosphere, and attachment to epidermal and root hair cells.
2. Characteristic curling of the root hairs and invasion of the bacteria to form an infection thread.
3. Nodule initiation and development in the root cortex. This stage is concurrent with stage 1.
4. Release of the bacteria from the infection thread and their differentiation as specialized nitrogen-fixing cells (Fig. 45.).

11.6. ábra - Figure 45. Schematic diagram of the infection process leading to nodule formation

(A) Rhizobia colonize the soil in the vicinity of the root hair in response to signals sent out from the host root. The rhizobia in turn stimulate the root hair to curl while, at the same time, sending mitogenic signals that stimulate cell division in the root cortex. (B) Rhizobia invade the root by digesting the root hair cell wall and forming an infection thread. The rhizobia continue to multiply as the infection thread elongates toward the root cortex. (C) The infection thread branches to penetrate numerous cortical cells as a visibly evident nodule develops on the root. The final stage (not shown) is the release of rhizobia into the host cells and the activation of the nitrogen-fixing machinery.

12. 11.12 N Deficiency
In order to achieve efficient growth, development and reproduction, plants require adequate, but not excessive, amounts of N. Therefore, low soil N availability or a decline in root uptake capacity will negatively affect plant productivity and ecological competitiveness. Nitrogen-deficient plants are typically stunted, with narrow leaves. Chlorosis caused by N deficiency typically begins in the older leaves as N is remobilized to younger leaves. At the field scale, N-deficient crops appear pale green or even yellow. The canopy height is lower and, in grasses, tillering as well as the number of seeds per inflorescence are reduced compared to plants growing with adequate N.

With temporary N starvation in the root medium, plants display a two-phase response. In the first phase, the leaf elongation rate is reduced without affecting photosynthesis. Root growth is maintained or even stimulated by transport of assimilated carbon to the roots, which results in a lower shoot/root biomass ratio (Fig. 46.).

11.7. ábra - Figure 46. Schematic representation of shoot and root growth in cereal plants with increasing N supply

Concomitantly, N compounds, particularly nitrate, are mobilized in order to maintain N metabolism and the capacity to take up nitrate from the soil is increased. In the second phase, upon continued N starvation, the breakdown of leaf nucleic acids and proteins is triggered. This is usually associated with leaf senescence. The breakdown of Rubisco leads to a decrease in the maximum photosynthetic capacity of the plant, ultimately inhibiting whole plant growth.

Plants have evolved multifaceted strategies to respond to variations in N availability in the soil, i.e. metabolic, physiological and developmental adaptations, which, in part, depend on changes in gene expression. The expression of many genes is changed within minutes in response to nitrate concentrations. In Arabidopsis, N deprivation or limitation leads to a coordinated repression of genes involved in photosynthesis, chlorophyll synthesis, plastid protein synthesis, while genes involved in secondary metabolism and protein degradation are induced.

13. 11.13 Agricultural and ecosystem productivity is dependent on nitrogen supply

In terms of quantity, nitrogen is the fourth most abundant element in plants and is the most abundant mineral element. On a dry-weight basis, herbaceous plant material typically contains between 1 and 4 percent nitrogen, mostly in the form of protein. At the same time, the availability of nitrogen in the soil may be limited by a number of environmental factors, such as temperature, oxygen, water status, and pH, which influence the activity of microorganisms responsible for nitrogen fixation, nitrification, and ammonification.

Moreover, a substantial quantity of nitrogen is removed each year with the harvested crop. It is not too surprising, then, that crop growth is most often limited by nitrogen supply. In agricultural situations, the application of nitrogen fertilizers overcomes environmentally imposed nitrogen limitation. Most crops respond to applied nitrogen with increases in yield. At sufficiently high application rates, factors other than nitrogen become limiting and there is no further gain. At even higher application rates, yield may decline slightly, but this is probably due to excess salt in the soil rather than some form of nitrogen toxicity. Throughout North America, corn is the leading consumer of nitrogen fertilizer and farmers typically apply 100 to 150 kgN ha−1 each growing season. During its early rapid growth phase, a well-irrigated stand of corn will take up as much as 4 kg of nitrogen ha−1 day−1. The use of such large amounts of nitrogen fertilizers is also costly in terms of
11. Nitrogen metabolism in plants

Energy. It has been estimated, for example, that fully one-third of the energy cost of a corn crop is accounted for by the production and distribution of nitrogen fertilizers. Without continued application of fertilizers, yields of nonleguminous crops traditionally decline over a period of years. In a few situations where records have been kept, yields on plots from which nitrogen fertilizers have been withheld will eventually stabilize at a lower level that can be sustained indefinitely. Sustained low yields are possible because the extraction of nitrogen (and other nutrients) from the soil is balanced against replenishment from all sources, including rainfall, irrigation water, dust, and weathering of parent rock.

The role of nitrogen in natural ecosystems is much more difficult to define, in part because the level of inputs is very low relative to the total nitrogen pool and much of the nitrogen is recycled. Still, it is generally agreed that nitrogen is limiting in most natural ecosystems just as it is in agriculture. In forest ecosystems, approximately two-thirds of the annual nitrogen input is contributed by nitrogen fixation, while the other third is believed to be derived primarily from atmospheric sources: either through rainfall or dry deposition of nitrogen oxides. A recent study has shown that, on average, close to half of the incoming fixed nitrogen is retained in the canopy. This should not be surprising since the photosynthetic apparatus present in the leaves of the canopy contains the bulk of the assimilated organic nitrogen in form of proteins such as Rubisco, the most abundant protein in nature, the major light harvesting polypeptides present in thylakoid membranes, and of course, the pigments, chlorophyll a and chlorophyll b, which are also rich in nitrogen. This implies that foliar absorption of nitrogen could play a significant role in nitrogen uptake by forest species. This seems particularly true of trees growing at high elevations, which are frequently bathed in cloud cover, or trees that grow near urban industrialized areas. Except in mature, slowly growing forests, most of the nitrogen is taken up and either retained in the canopy or held in long-term storage in the litter on the forest floor where it is slowly recycled. The nitrogen content of the litter is slowly leached into the soil by rain and surface water or is broken down into simpler compounds by a variety of soil bacteria, fungi, earthworms, and other decomposing organisms. The final step in the breakdown is mineralization, or the formation of inorganic nitrogen from organic nitrogen.

Mineralization is largely due to the process of ammonification described earlier. Mineralization is invariably accompanied by immobilization, or the retention and use of nitrogen by the decomposing organisms. Availability of litter nitrogen to plants depends above all on net mineralization, or the extent to which mineralization exceeds immobilization.

The balance between mineralization and immobilization and oxidation of the mineralized NH4+—nitrogen by nitrifying bacteria is regulated by environmental parameters. Principal among these are temperature, pH, soil moisture, and oxygen supply. The optimum temperature for nitrification generally falls between 25°C and 35°C, although climatic adaptations of indigenous nitrifying bacteria to more extreme temperatures have been demonstrated. In one study, for example, the optimum temperature for nitrification in soils of northern Australia was 35°C, in Iowa (U.S.) it was 30°C, and in Alberta (Canada), 20°C. As noted earlier, soil pH is a major limiting factor in the growth of nitrifying bacteria. The growth of Nitrobacter is probably inhibited by ammonium toxicity at high pH (7.5 and above), while aluminum toxicity is suspected as the cause of limited nitrification in acid soils. Soil moisture and oxygen supply go hand-in-hand—little nitrification occurs in water-saturated soils because of the limited O2 supply.

At the other extreme, the rate of nitrification declines with decreasing soil water potential (_/soil) below about ~0.03 to ~0.04 MPa. The relative significance of nitrification in the nitrogen cycle of natural ecosystems is not altogether clear. Most studies indicate a relatively minor role, since little if any surplus NO3- is found in the soil or streams of most undisturbed ecosystems. Experimental deforestation, however, leads to a rapid rise (as much as 50-fold) in the levels of NO3- in stream water. NO3- levels gradually returned to normal only as the vegetation began to regrow. These results suggest that nitrification is a significant source of nitrogen, but rapid uptake of NO3- by plants is an important factor in maintaining low levels of NO3- in the soil solution.

Trees and other plants also tend to conserve a large proportion of their nitrogen, withdrawing nitrogen from the leaves and flowers before they are shed and placing it in storage in the roots and stem tissues. Between one-third and two-thirds of a plant’s nitrogen may be conserved by such internal cycling. In the case of deciduous trees, for example, this stored nitrogen offers a degree of nutritional independence from the often nitrogen-poor soil during the flush of growth in early spring.

14. 11.14 The most important amino acids synthesis

14.1. 11.14.1 Essential and Nonessential Amino Acids
11. Nitrogen metabolism in plants

Nonessential amino acids are those that are synthesized by mammals, while the essential amino acids must be obtained from dietary sources. Why would an organism evolve in such a way that it could not exist in the absence of certain amino acids? Most likely, the ready availability of these amino acids in lower organisms (plants and microorganisms) obviated the need for the higher organism to continue to produce them. The pathways for their synthesis were selected out. Not having to synthesize an additional ten amino acids (and regulate their synthesis) represents a major economy, then. Nevertheless, it remains for us to become familiar with the synthetic pathways for these essential amino acids in plants and microorganisms, and it turns out that they are generally more complicated that the pathways for nonessential amino acid synthesis and they are also species-specific.

14.2. 11.14.2 Synthesis of Nonessential Amino Acids

Ignoring tyrosine (as it's immediate precursor is phenylalanine, an essential amino acid), all of the nonessential amino acids (and we will include arginine here) are synthesized from intermediates of major metabolic pathways. Furthermore, the carbon skeletons of these amino acids are traceable to their corresponding α-ketoacids. Therefore, it could be possible to synthesize any one of the nonessential amino acids directly by transaminating its corresponding α-ketoacid, if that ketoacid exists as a common intermediate. A "transamination reaction", in which an amino group is transferred from an amino acid to the α-carbon of a ketoacid, is catalyzed by an aminotransferase.

Three very common α-ketoacids can be transaminated in one step to their corresponding amino acid:

Pyruvate (glycolytic end product) --> alanine
Oxaloacetate (citric acid cycle intermediate) --> aspartate
α-ketoglutarate (citric acid cycle intermediate) --> glutamate

The individual reactions are (Fig. 47.):

11.8. ábra - Figure 47. The glutamine synthesis
Asparagine and glutamine are the products of amidations of aspartate and glutamate, respectively. Thus, asparagine and glutamine, and the remaining nonessential amino acids are not directly the result of transamination of α-ketoacids because these are not common intermediates of the other pathways. Still, we will be able to trace the carbon skeletons of all of these back to an α-ketoacid. I make this point not because of any profound implications inherent in it, but rather as a way to simplify the learning of synthetic pathways of the nonessential amino acids.

Aspartate is transaminated to asparagine in an ATP-dependent reaction catalyzed by asparagine synthetase, and glutamine is the amino group donor (Fig. 48):  

**11.9. ábra - Figure 48. The synthesis of asparagine**

The synthesis of glutamine is a two-step one in which glutamate is first "activated" to a glutamylphosphate intermediate, followed by a reaction in which NH3 displaces the phosphate group.
So, the synthesis of asparagine is intrinsically tied to that of glutamine, and it turns out that glutamine is the amino group donor in the formation of numerous biosynthetic products, as well as being a storage form of $\text{NH}_3$. Therefore, one would expect that glutamine synthetase, the enzyme responsible for the amidation of glutamate, plays a central role in the regulation of nitrogen metabolism. We will now look into this control in more detail, before proceeding to the biosynthesis of the remaining nonessential amino acids.

You have previously studied the oxidative deamination of glutamate by glutamate dehydrogenase, in which $\text{NH}_3$ and $\alpha$-ketoglutarate are produced. The $\alpha$-ketoglutarate produced is then available for accepting amino groups in other transamination reactions, but the accumulation of ammonia as the other product of this reaction is a problem because, in high concentrations, it is toxic. To keep the level of $\text{NH}_3$ in a controlled range, a rising level of $\alpha$-ketoglutarate activates glutamine synthetase, increasing the production of glutamine, which donates its amino group in various other reactions.

The activity of the enzyme is controlled by 9 allosteric feedback inhibitors, 6 of which are end products of pathways involving glutamine:

- histidine
- tryptophan
- carbamoyl phosphate (synthesized from carbamoyl phosphate synthetase II)
- glucosamine-6-phosphate
- AMP
- CTP

The other three effectors are alanine, serine and glycine, which carry information regarding the cellular nitrogen level.

15. 11.15 DNA, RNA and Protein Synthesis

15.1. 11.15.1 Structure and function of DNA

DNA molecules are incredibly long, but also very thin. One DNA molecule from the chromosome of a mammal may be about 1 m long when unraveled. However, it has to fit in a nucleus of some 5-6 orders of magnitude smaller and is folded up in chromosomes in a highly organized manner. DNA is a linear polymer that is composed of four different building blocks, the nucleotides. It is in the sequence of the nucleotides in the polymers where the genetic information carried by chromosomes is located. Each nucleotide is composed of three parts: (1) a nitrogenous base known as purine (adenine (A) and guanine (G)) or pyrimidine (cytosine (C) and thymine (T)); (2) a sugar, deoxyribose; and (3) a phosphate group (see pp. 20-22 of Molecular Biotechnology for molecular structures of DNA and its components). The nitrogenous base determines the identity of the nucleotide, and individual nucleotides are often referred to by their base (A, C, G, or T). One DNA strand can be up to several hundred million nucleotides in length. $\text{T}$ can form a hydrogen bond with $\text{A}$, and $\text{C}$ with $\text{G}$; two DNA strands wind together in an antiparallel fashion in a double-helix (Fig.49.).

11.10. ábra - Figure 49. Double-helix structure of DNA
Inside the cell, the DNA acts like an "instruction manual": in its sequence, it provides all the information needed to function, but the actual work of translating the information into a medium that can be used directly by the cell is done by RNA, ribonucleic acid. The structural difference with DNA is that RNA contains a -OH group both at the 2' and 3' position of the ribose ring, whereas DNA (which stands, in fact, for deoxy-RNA) lacks such a hydroxy group at the 2' position of the ribose. The same bases can be attached to the ribose group in RNA as occur in DNA, with the exception that in RNA thymine does not occur, and is replaced by uracil, which has an H-group instead of a methyl group at the C-5 position of the pyrimidine. The RNA has three functions: (a) it serves as the messenger that tells the cell (the ribosomes) what protein to make (messenger RNA; mRNA); (b) it serves as part of the structure of the ribosome, the protein/RNA complex that synthesizes proteins according to the information presented by the mRNA (ribosomal RNA; rRNA); and (c) it functions to bring amino acids (the constituents of the proteins) to the ribosome when a specific amino acid "is called for" by the information on the mRNA to be put into the protein that is being synthesized; this RNA is called transfer RNA (tRNA).

An important point of emphasis should be that all vegetative cells of one organism contain the same genetic information. Upon division, each daughter cell obtains an "exact" copy of the DNA of the parent. However, the specific genes that are expressed at specific times may be very different between different tissues. These differences in gene expression allow for the regulation of development of the organism, and for the development of different tissues. For the most part, DNA-binding proteins (encoded by the DNA) play an important role in the regulation of expression of genes encoded on the DNA.

15.2. 11.15.2 RNAs

The messenger RNA (mRNA) serves as an intermediate between DNA and protein. Parts of the DNA are "transcribed" into transcripts (single-stranded RNA molecules) that are processed to mRNA. In prokaryotes the transcript generally does not need to be processed, and can serve as mRNA right away. Transcription starts at a specific site on the DNA called a promoter. Each gene or operon has its own promoter(s). Transcription ends at a terminator sequence on the DNA. The transcripts usually are 300-50,000 nucleotides long, and contain the information to make protein. In eukaryotes (organisms with cells containing a nucleus; in fact, any higher organism) generally the transcripts needs to be processed before they can serve as a blueprint for a protein. The processing involves the removal of intervening sequences (introns) in the gene. The introns may be anywhere between 50 and 10,000 nucleotides in length. The coding regions of the mRNA are called exons. There may be up to 100 introns in a single gene. The introns are spliced out by small ribonucleoprotein particles (consisting of RNA and protein), which appear to pull the two ends of the intron together. However, there are also introns that splice out without the need of a protein: the RNA sequence itself appears to contain sufficient information to know where to splice out the intron. In addition to the removal of introns, a poly-A sequence is added to the 3’ end of the transcript. The processed transcript is the mRNA, and the information in the mRNA can be used to be "translated" into a protein of specific sequence. However, in prokaryotes introns are rare and mRNA generally does not get processed before translation.
The intron splicing process provides an opportunity to increase the amount of usable genetic information without increasing the genome size of the organism: Alternative splicing of a particular transcript can occur. Alternative splicing means that introns may be recognized in different ways in different molecules of the same primary transcript, and the result is that one gene can give rise to different mRNAs and thereby to different proteins. Note that this process is largely limited to eukaryotes as introns in prokaryotes are rare.

Ribosomal RNAs (rRNAs) are essential components of an important part of the protein synthesis machinery: the ribosomes. In addition to rRNA, there are some 70 different proteins in a ribosome. There are hundreds of copies of rRNA genes per genome, thus making the production of lots of rRNA possible. There are four different rRNAs, each with a different size. Each ribosome contains one molecule of each of the four rRNA types. In prokaryotes, ribosomes bind to the mRNA close to the translation start site. This ribosome binding site is referred to as the Shine-Dalgarno sequence or as the ribosome recognition element. In eukaryotes, ribosomes bind at the 5' end of the mRNA and scan down the mRNA until they encounter a suitable start codon.

Transfer RNA (tRNA) carries amino acids to the ribosomes, to enable the ribosomes to put this amino acid on the protein that is being synthesized as an elongating chain of amino acid residues, using the information on the mRNA to "know" which amino acid should be put on next. For each kind of amino acid, there is a specific tRNA that will recognize the amino acid and transport it to the protein that is being synthesized, and tag it on to the protein once the information on the mRNA calls for it.

All tRNAs have the same general shape, sort of resembling a clover leaf. Parts of the molecule fold back in characteristic loops, which are held in shape by nucleotide-pairing between different areas of the molecule. There are two parts of the tRNA that are of particular importance: the aminoacyl attachment site and the anticodon. The aminoacyl attachment site is the site at which the amino acid is attached to the tRNA molecule. Each type of tRNA specifically binds only one type of amino acid. The anticodon (three bases) of the tRNA base-pairs with the appropriate mRNA codon at the mRNA-ribosome complex. This temporarily binds the tRNA to the mRNA, allowing the amino acid carried by the tRNA to be incorporated into the polypeptide in its proper place. Thus, the sequence of the codon (three bases) in the mRNA dictates the amino acid to be put in in the protein at a specific site. The "dictionary" of codons coding for amino acids is called the genetic code. The three codons for which there is no matching tRNA (UAA, UGA, and UAG) serve as "stop-translation" signals at which the ribosome falls off (Fig. 50.).

11.11. ábra - Figure 50. The biosynthesis of proteins

15.3. 11. 15. 3. Protein synthesis

After having discussed DNA and the various RNAs, the stage has been set for protein synthesis. The basic reaction of protein synthesis is the controlled formation of a peptide bond between two amino acids. This reaction is repeated many times, as each amino acid in turn is added to the growing polypeptide. Protein synthesis starts when the mRNA binds to a small ribosomal subunit near a AUG sequence in the mRNA. The AUG codon is called start codon, since it codes for the first amino acid (a methionine) to be made of the protein. The AUG codon base-pairs with the anticodon of tRNA carrying methionine. A large ribosomal subunit binds to the complex, and the reactions of protein synthesis itself can begin. The aminoacyl-tRNA to be called for next is determined by the next codon (the next three bases) on the mRNA. Each amino acid is coded for by one or more
(up to six) codons. Of course, it would be more straightforward to have each amino acid coded for by only one codon, but nature appears to have chosen a more complex route. The reason for this in part is that there are 20 different amino acids, and $4 \times 4 \times 4 = 64$ different combinations possible in a codon. When the ribosome reaches one of the three codons for which there is no matching tRNA, the ribosome falls off and the synthesized protein is released. The degeneracy of the genetic code for certain amino acids could have a function in regulation of translation; any idea how? Amino acids represent quite a broad spectrum of different chemical structures (Fig. 51).

11.12. ábra - Figure 51. Summery of protein synthesis

![Diagram of protein synthesis](image)

15.4. 11.15.4 RNA editing

Over the last several years, it has become obvious that the sequence present in DNA does not always dictate literally the sequence of the protein. In a number of instances “RNA editing” has been observed (particularly in the small genomes present in mitochondria and chloroplasts), in which transcripts are chemically modified (for example, some Cs are changed to Us) by enzymes before translation takes place. Thus, the DNA sequence in such cases does not precisely correlate with the sequence of the gene product (the protein). One thus needs to compare sequences from DNA and protein (or from DNA and processed RNA) if one suspects that RNA editing can occur. The function of RNA editing has not been elucidated yet.

16. 11. 16. Literature


11. Nitrogen metabolism in plants


17. 11.17 Questins

1. Heavy fertilization of agricultural crops with nitrogen is a costly process, both economically and energetically. Is it feasible to produce crops without nitrogen fertilizers? If so, what would be the consequences with respect to yields?

2. What are ammonification, nitrification, and denitrification? What are their respective contributions to the nitrogen cycle?

3. Describe the process of rhizobial infection and nodule development in a legume root!

4. Describe the protein and DNA syntheis!
12. fejezet - 12. Growth and development, Plant hormones I.

Three terms routinely used to describe various changes that a plant undergoes during its life cycle are growth, differentiation, and development.

Development is an umbrella term, referring to the sum of all of the changes that a cell, tissue, organ, or organism goes through in its life cycle. Development is most visibly manifested as changes in the form of an organ or organism, such as the transition from embryo to seedling, from a leaf primordium to a fully expanded leaf, or from the production of vegetative organs to the production of floral structures.

Growth is a quantitative term, related only to changes in size and mass. For cells, growth is simply an irreversible increase in volume. For tissues and organs, growth normally reflects an increase in both cell number and cell size. It should be obvious that many parameters could be invoked to measure growth, dependent to some extent on the needs of the observer. Whatever the measure, however, all attempts to quantify growth reflect a fundamental understanding that growth is an irreversible increase in volume or size.

Differentiation refers to differences, other than size, that arise among cells, tissues, and organs. Differentiation occurs when cells assume different anatomical characteristics and functions, or form patterns. Differentiation begins in the earliest stages of development, such as, when division of the zygote gives rise to cells that are destined to become either root or shoot. Later, unspecialized parenchyma cells may differentiate into more specialized cells such as xylem vessels or phloem sieve tubes, each with a distinct morphology and unique function. Differentiation does not lend itself easily to quantitative interpretation but may be described as a series of qualitative, rather than quantitative, changes. Finally, although growth and differentiation are normally concurrent events, examples abound of growth without differentiation and differentiation without growth.

Differentiation is a two-way street and is not determined so much by cell lineage as by cell position with respect to neighboring cells. Thus, even though some plant cells may appear to be highly differentiated or specialized, they may often be stimulated to revert to a more embryonic form. This ability of differentiated cells to revert to the embryonic state and form new patterns without an intervening reproductive stage is called totipotency. Most living plant cells are totipotent—something akin to mammalian stem cells—and retain a complete genetic program even though not all of the information is used by the cell at any given time. In this sense, development does not reflect a progressive loss of genetic information, only the selective use of that information in order to achieve particular developmental ends.

Not all cells are totipotent. Highly specialized cells whose development has been locked in, such as by exceptionally thick and rigid secondary cell walls or severely modified protoplasts, are not capable of renewed differentiation. On the other hand, it is probable that all tissues contain at least some potentially totipotent cells - cells that have the morphogenetic potential of a zygote. Plant development proceeds in an orderly fashion because that potential is carefully limited. When those limitations are removed, totipotent cells simply revert to the zygotic state and begin the developmental program anew.

Plant growth is limited to discrete regions where the cells retain the capacity for continued cell division. These regions are called meristems (Gk. merizeim, to divide). Two such regions are the apical meristems located at the tips of roots and stems. These regions of active cell division are responsible for primary growth, or the increase in the length of roots and stems. The tip of the root is covered by a root cap, which provides mechanical protection for the meristem as the root grows through the abrasive soil medium. The root cap also secretes polysaccharides, which form a muclaginous matrix called mucigel. Mucigel lubricates the root tip as it moves through the soil. The root cap along with its coating of mucigel is also involved in perception of gravity by roots. The root apical meristem (RAM) is a cluster of dividing cells located at the tip of the root just behind the root cap (Fig.52).

12.1. ábra - Figure 52. Schematic diagram of a young Arabidopsis root tip showing the principal regions of the root apical meristem
Each time a cell in the meristem divides, one of the two daughter cells will be retained to continue cell division while the second daughter cell proceeds to elongate, thus increasing the length of the root and pushing the root tip through the soil. In the center of the meristem is a region of slowly dividing cells called the quiescent zone. Cell divisions responsible for new tissues in the elongation root and regeneration of the root cap take place around the periphery of the quiescent zone. The shoot apical meristem (SAM) is structurally more complex than the root apical meristem. This is understandable because in addition to producing new cells that elongate and extend the length of the axis of the shoot, the shoot apical meristem must also form primordia that give rise to lateral organs such as leaves, branches, and floral parts. At the same time it must perpetuate itself by maintaining a small population of undifferentiated, dividing cells. Similar to the root apical meristem, each time a cell divides in the SAM, one daughter cell is left behind to elongate and move the shoot apex forward while the other daughter cell remains within the meristem to continue dividing.

Tissues that are derived directly from the root and shoot apical meristems are called primary tissues. The stems and roots of woody plants, however, grow in diameter as well. An increase in diameter results from the activity of a meristem called the vascular cambium. Tissues laid down by the vascular cambium are called secondary tissues, so the vascular cambium is responsible for secondary growth. The primary tissue of roots and shoots contains a central core of vascular, or conducting, elements. Characteristically, the xylem lies toward the center of the vascular core and the phloem lies at the outer edge of the core (Fig. 53).

**12.2. ábra - Figure 53.**

*The vascular cambium arises between the primary phloem and primary xylem and adds new, or secondary, xylem cells to the inside and new, or secondary, phloem cells to the outside. This is repeated annually to add girth to the stem. The secondary xylem develops heavy, lignified walls and becomes the woody tissue of the stem.*
The phloem is a soft tissue and each year’s new growth crushes the previous year’s phloem. When you peel the ‘‘bark’’ off a young stem, the vascular cambium is where the tissues separate.

The vascular cambium develops between the xylem and phloem and produces new xylem toward the inside and new phloem toward the outside. Because of its heavy cell walls and eventual lignification, xylem is a rigid and long-lasting tissue that eventually occupies the bulk of most woody stems or trunks. Phloem is a more fragile tissue and with each year’s new growth the previous year’s cells tend to be pushed outward and crushed. As a result, the xylem continues to transport water and minerals for several years, but a large tree seldom has more than one-year’s worth of functioning phloem.

1. 12.1 Plant growth regulators

The plant growth regulators (PGRs) are small, simple molecules of diverse chemical composition. They could be indole compounds (indole-3-acetic acid, IAA); adenine derivatives (N6-furfurylamino purine, kinetin), derivatives of carotenoids (abscisic acid, ABA); terpenes (gibberellic acid, GA3) or gases (ethylene, C2H4). Plant growth regulators are variously described as plant growth substances, plant hormones or phytohormones in literature.

The PGRs can be broadly divided into two groups based on their functions in a living plant body. One group of PGRs are involved in growth promoting activities, such as cell division, cell enlargement, pattern formation, tropic growth, flowering, fruiting and seed formation. These are also called plant growth promoters, e.g., auxins, gibberellins and cytokinins. The PGRs of the other group play an important role in plant responses to wounds and stresses of biotic and abiotic origin. They are also involved in various growth inhibiting activities such as dormancy and abscission. The PGR abscisic acid belongs to this group. The gaseous PGR, ethylene, could fit either of the groups, but it is largely an inhibitor of growth activities.

2. 12.2 Auxin

2.1. 12.2.1 The Discovery of Auxins

In the late 1870s, Charles Darwin and his son Francis were one of the earliest scientists who studied phototropism (the growth of stems and leaves toward light).

The Darwins studied coleoptiles (the protective sheath around the embryonic shoot in grass seeds) of canary grass and oats. They discovered that both plants grow toward the light source.

The Darwins followed up this discovery with these experiments:

1. The tips of coleoptiles were covered with a metal foil. This blocked the incoming light and the coleoptiles did not grow toward light. When the foil was removed, they grew toward the light.

2. The growing region of the coleoptiles rather than their tips was covered and they discovered that the coleoptiles grew toward the light. The conclusion was made that the growth of coleoptiles toward light was controlled by the tip of the coleoptile.

The Darwins suggested that, “phototropism was due to an ‘influence’ produced in the tip of a coleoptile that moved to the growing region, where it caused the coleoptile to grow toward light.” Their discovery helped later scientists discover plant hormones.

In 1913, Peter Boysen-Jensen further developed on the Darwins experiments (Fig. 54.):

1. He cut off the tip of a coleoptile and noticed that it stopped its growth, which showed that something within the tip of the coleoptile controlled growth.

2. He then separated the tip from the coleoptile with a tiny piece of agar. He observed that the coleoptiles grew and curved toward the light. He concluded that the tips of the coleoptiles did not have to be in their normal position to affect growth, and the chemical that controlled phototropism moved through agar, therefore it was a water-soluble chemical.
3. He replaced the agar block with butter. Since water is insoluble in butter, any water-soluble chemical from the tip could not move through the butter into the growing region. He observed that there was no growth, and from this concluded that the chemical was water-soluble.

4. To test if the signal was electrical he replaced the agar blocks with pieces of Pt foil, and there was no growth. Therefore, the signal was chemical rather than electrical.

In 1918, Arpad Paal continued on with Boysen-Jensen's experiments to identify the chemical. He studied coleoptiles grown in the dark:

1. He cut off the tips of coleoptiles grown in the dark, and placed them on one side of the cut surface. These curved away from the side onto which the tips were placed, despite them being Paal concluded that the coleoptile's tip produces something that travels down and stimulates growth, and that light causes the accumulation of the chemical on the shaded side of the coleoptile.

Frits Went finalized all these experiments (1926):

He cut off the tips and placed the cut surfaces onto agar. The tips were removed after an hour and the agar was placed on the cut tips of the coleoptiles grown in the dark.

Went's different experiments and results:

1. Cut off coleoptiles & without agar blocks, did not grow. This confirmed that the tips produced something essential for growth.

2. Agar blocks that contacted cut tips were placed on the center of the cut off coleoptiles and they grew straight up. Therefore, the chemical diffused into the agar from the coleoptile tips, and stimulated their growth.

3. Agar blocks that did not contact the cut tips of coleoptiles did not show any response. Therefore, nothing in the agar caused growth of the coleoptile.

4. Agar blocks that had contacted the cut tips when placed on one side of the cut off coleoptiles, curved away from the agar blocks. This confirmed that the agar blocks had a chemical that stimulated growth of coleoptiles.

Went concluded that the phototrophic response was due to a chemical coming from the coleoptile's tip. He named this chemical auxin, which comes from a Greek word meaning “to grow.”

12.3. ábra - Figure 54. Examination of auxin based on Darwin and Boysen-Jensen
2.2. 12.2.2 The principal auxin in plants in indole-3-acetic acid (IAA)

Although a large number of compounds have been discovered with auxin activity, indole-3-acetic acid (IAA) is the most widely distributed natural auxin (Fig.55). In addition to IAA, several other naturally occurring indole derivatives are known to express auxin activity, including indole-3-ethanol, indole-3-acetaldehyde, and indole-3-acetonitrile. However, these compounds all serve as precursors to IAA and their activity is due to conversion to IAA in the tissue. The initial discovery of IAA in plants and recognition of its role in growth and development stimulated the search for other chemicals with similar activity. The result has been an array of synthetic chemicals that express auxin-like activity. One of these chemicals was indole-3-butyric acid (IBA) (Fig.55). More recently, IBA has been isolated from seeds and leaves of maize and several other species. A chlorinated analog of IAA (4-chloroindoleacetic acid, or 4-chloroIAA; II, Figure x) has also been reported in extracts of legume seeds and a closely related, naturally occurring aromatic acid, phenyl acetic acid (PAA) (Fig.55) has recently been reported to have auxin activity. Because IBA, 4-chloroIAA, and PAA have now been isolated from plants, are structurally similar to IAA, and elicit many of the same responses as IAA, there is a strong argument for considering them natural hormones. However, it is not yet clear whether they are active on their own or whether they are first converted to IAA. Chemically, the single unifying character of molecules that express auxin activity appears to be an acidic side chain on an aromatic ring.

12.4. ábra - Figure 55. The chemical structures of some naturally occurring and synthetic auxins
Indole-3-acetic acid (I) is believed to be the active auxin in all plants. Phenylacetic acid (III) is widespread and two others, 4-chloroindole-3-acetic acid and indole-3-butyric acid, have been identified in plant extracts. The latter three induce auxin responses when applied exogenously, but probably act via conversion to IAA. Structures VI, VII, and VIII are active herbicides.

The amount of IAA present will depend on a number of factors, such as the type and age of tissue and its state of growth. In vegetative tissues, for example, the amount of IAA generally falls in the range between 1 μg and 100 μg (5.7 to 570 nanomoles) kg⁻¹ fresh weight, but in seeds it appears to be much higher. In one study, it was estimated that the endosperm of a single maize seed four days after germination contains 308 picomoles (pmole = 10⁻¹² mole) of IAA. At the same time, the maize shoot contained 27 pmoles of IAA and required an estimated input of approximately 10 pmoles of IAA hr⁻¹ in order to support its growth. The high level of IAA in the seed apparently serves to support the rapid growth of the young seedling when the seed germinates.

2.3. 12.2.3 Synthesis of IAA

Since the 1930s, when K. V. Thimann first observed the synthesis of IAA in the mold Rhizopus suinus, which had been fed the amino acid tryptophan, the conversion of tryptophan to IAA has been studied in vivo in more than 20 different plant species and in vitro with at least 10 different cell-free enzyme preparations. The synthesis of IAA is normally studied by feeding plants tryptophan carrying a radioactive label, usually carbon (14C) or tritium (3H), and examining the radioactivity of subsequently isolated IAA or its intermediates. Feeding experiments are complicated by several factors and the results must always be approached with caution. For example, radiolabeled tryptophan can apparently undergo radiochemical decomposition, thus giving rise to IAA by nonenzymatic reactions. In addition, the pool size of tryptophan (also a precursor for protein synthesis) is
very large relative to that of IAA and there is little data on the actual quantity of IAA synthesized. Finally, care must be taken to ensure that experiments are conducted under sterile conditions, since many microorganisms readily convert tryptophan to IAA. While these complications make it difficult to ascertain the exact pathway that functions in vivo, the available evidence clearly establishes that plants are able to synthesize IAA from tryptophan. In most plants, synthesis of IAA occurs in three steps, beginning with the removal of amino group on the tryptophan side chain. The product is indole-3-pyruvic acid (IPA) (Fig.56).

12.5. ábra - Figure 56. Pathway for tryptophan-dependent biosynthesis of indole-3-acetic acid. Hedden P., Phillips A. L.: Manipulation of hormone biosynthetic genes in transgenic plants. In: Current Opinion in Biotechnology 11 (2) 130-137.

This reaction is catalyzed by tryptophanamino transferase, a widely distributed multispecific enzyme that appears to well to remove amino groups from structural analogs of tryptophan such as phenylalanine and tyrosine. The second step is the decarboxylation of IPA to form indole-3-acetaldehyde (IAAld). The enzyme that catalyzes this step, indole-3-pyruvate decarboxylase, has been described in several plant tissues and cell-free extracts. Finally, IAAld is oxidized to IAA by a NAD-dependent indole-3-acetaldehyde oxidase. The presence of this enzyme has been demonstrated in a number of tissues, including oat coleoptile. IAAld may also be reversibly reduced to indole-3-ethanol. Indole-3-ethanol is active in bioassays using stem sections, but this is probably due to its conversion to IAA in the tissue. Finally, IAA can be reversibly converted to IBA by the enzyme indole-3-butyric acid synthase.

There is some evidence for alternate biosynthetic pathways involving intermediates other than IPA, but the burden of biochemical evidence indicates that the IPA pathway is the principal pathway for the synthesis of IAA from tryptophan in higher plants. Although IAA-deficient mutants might be expected to provide further useful information, none have been identified to date. This is perhaps because an IAA deficiency would probably be lethal.

2.4. 12.2.4 Polar transport of auxin

Auxin transport has naturally been studied almost exclusively in young seedlings, where synthesis takes place in the actively proliferating tissues. From these regions, there appears to be a steady stream of auxin flowing down the shoot into the root. At least in Arabidopsis seedlings, some of this stream apparently moves down a concentration gradient in the phloem. A significant portion, however, moves through a complex, highly regulated polar transport mechanism. Polar transport was originally described based on preferential movement either up or down in grass coleoptiles, stems, and roots (Fig.57). When movement is away from the morphological apex toward the morphological base of the transporting tissue, the direction of movement is described as basipetal. Movement in the opposite direction, toward the morphological apex, is referred to as acropetal. When a stem or coleoptile section is inverted, as shown in Figure x, the original direction of movement is maintained. However, as more is learned about auxin transport, the more evident it becomes that directed auxin transport may be lateral as well as up and down. Polar transport of auxin in shoots tends to be predominantly basipetal at a velocity somewhere between 5 and 20mm hr⁻¹. Acropetal transport in shoots is minimal. In roots, on the other hand, there appear to be two transport streams. An acropetal stream, arriving from the shoot, flows through xylem parenchyma cells in the central cylinder of the root and directs auxin
toward the root tip. A basipetal stream then reverses the direction of flow, moving auxin away from the root tip, or basipetally, through the outer epidermal and cortical cell files.

12.6. ábra - Figure 57. Polarity in auxin transport in an oat coleoptile segment. The donor block contains 14C-IAA.

Regardless of the orientation of the segment, translocation of the radio-labeled IAA is always from the morphologically apical end (A) to the morphologically basal end (B) of the segment.

2.5. 12.2.5 Auxin controls the growth of axillary buds

As a shoot continues to grow and the apical meristem lays down new leaf primordia, small groups of cells in the axil (the angle between the stem and the leaf primordium) of the primordia become isolated from the apical meristem and produce an axillary bud. In some cases, such as the bean (Phaseolus), the bud continues to grow, although at a much slower rate than the apical bud. In many plants, however, mitosis and cell expansion in the axillary bud is arrested at an early stage and the bud fails to grow. It has been known for some time that removal of the shoot apex, a common horticultural technique for producing bushy plants, stimulates the axillary bud to resume growth (Fig.58).

12.7. ábra - Figure 58. Auxin suppresses the growth of axillary buds in bean (Phaseolus vulgaris) plants
12. Growth and development, Plant hormones I.

A) The axillary buds are suppressed in the intact plant because of apical dominance. B) Removal of the terminal bud releases the axillary buds from apical dominance (arrows). C) Applying IAA in lanolin paste (contained in the gelatin capsule) to the cut surface prevents the outgrowth of the axillary buds. (Photos ©M. B. Wilkins.)

Apparently the apical bud is able to exert a dominant influence that suppresses cell division and enlargement in the axillary bud. For this reason, the phenomenon of coordinated bud development is known as apical dominance. Shortly after auxin was first discovered, K. V. Thimann and F. Skoog questioned whether there might be a relationship between the capacity of the shoot tip to release auxin and its capacity to suppress axillary bud development—in other words, is apical dominance controlled by auxin? Thimann and Skoog tested this idea by decapitating broad bean (Vicia faba) plants and applying auxin to the cut stump. Axillary bud development remained suppressed in the presence of auxin. Since this initial demonstration, the capacity of auxin to substitute for the shoot tip in maintaining apical dominance has been confirmed repeatedly. How does auxin from the shoot apex suppress axillary bud development? The most widely accepted theory holds that the optimum auxin concentration for axillary bud growth is much lower than it is for the elongation of stems. The stream of auxin flowing out of the shoot apex toward the base of the plant is thought to maintain an inhibitory concentration of auxin at the axillary bud. Removal of this auxin supply by decapitation reduces the supply of auxin in the region of the axillary bud and thereby relieves the bud of inhibition. More direct evidence for the role of auxin transport is offered by the observation that inhibitors of auxin transport (TIBA and NPA) stimulate release of buds from dominance when applied to the stem between the shoot apex and the bud. In addition, lines of tomato that exhibit prolific branching (that is, the absence of apical dominance) also fail to export radioactively labeled IAA from the shoot apex.

3. 12.3 Literature


4. 12.4 Questions

1. Distinguish between growth, differentiation, and development. Can you give examples of each?

2. Describe the significance of meristems!

3. Auxin transport is uniquely polar in character. How is this directional transport accomplished?
4. Review the synthesis of IAA from tryptophan. Do all plants synthesize IAA from tryptophan?

5. What happens when the auxin concentration in some structures of the plant is over the action range of the hormone?
13. fejezet - 13. Plant hormones II.

1. 13.1 Gibberellins

Gibberellins are a large class of molecules. In fact, more than 135 have now been identified in higher plants and fungi and additional members are added almost every year. Only a few of these are biologically active in their own right. The others are either intermediates in the biosynthetic pathway or products of inactivation. It is worth noting, however, that the number of gibberellins found in any one species or organ may be very small and the number of active gibberellins smaller yet. It is believed, for example, that GA1 and GA4 are the principal naturally occurring, active gibberellins in higher plants.

All gibberellins are diterpenes based on the 20-carbon ent-gibberellane structure (Fig.59).

13.1. ábra - Figure 59. The ent-gibberellane skeleton and chemical structures of selected active and inactive gibberellins. GA8 is inactive because of the addition of the hydroxyl group in the 2 position.

A little more than one-third of the gibberellins characterized to date have retained the full complement of 20 carbon atoms and are known as C20-gibberellins. The others have lost carbon atom number 20 and are consequently known as C19-gibberellins. With a complex ring structure and the number of possible substitutions on 19 or 20 carbon atoms, it is not difficult to see how there could be such a large number of gibberellins.

Gibberellins that are demonstrated to be naturally occurring and that have been chemically characterized are assigned an “A” number. This number does not imply chemical relationships; it is assigned roughly in order of discovery. A C20-gibberellin commonly known as gibberellic acid was one of the first to be isolated and characterized. Because GA3 is readily extracted from fungal cultures, it is also the most common commercially
available form. GA1, GA3, and GA4, all of which promote vegetative growth, are the most active gibberellins and, consequently, the most widely used in gibberellin research.

### 1.1. 13.1.1 Discovery of gibberellins

During the late nineteenth and early twentieth centuries, Japanese rice farmers grew concerned about a disease that seriously reduced the yield of their crops. Plants infected with the bakanae (‘‗foolish seedling‘’) disease exhibited weak, elongated stems and produced little or no grain. Japanese plant pathologists, interested in developing means for controlling the disease, soon established a connection with the presence of a fungus, Gibberella fujikuroi. In 1926, E. Kurosawa reported the appearance of symptoms of the disease in uninfected rice plants that had been treated with sterile filtrates from cultures of this fungus. By 1938, Japanese investigators had isolated and crystallized the active material, which they called gibberellin after the genus name for the fungus.

Gibberellin did not come to the attention of Western plant physiologists until after the 1939–1945 war, when two groups - one headed by Cross in England and one by Stodola in the United States - isolated and chemically characterized gibberellic acid from fungal culture filtrates. At the same time, Japanese workers isolated three gibberellins, which they named gibberellin A1, gibberellin A2, and gibberellin A3. Gibberellin A3 proved to be identical with gibberellic acid. The known effect of gibberellins on rice and several other plant systems indicated that similar substances might be present in higher plants as well. The first higher-plant gibberellin to be characterized was isolated from immature seeds of runner bean (Phaseolus coccineus) and found to be identical with gibberellin A1. Since then, gibberellins have been shown to be ubiquitous in higher plants.

### 1.2. 13.1.2 Gibberellins affect many aspects of plant growth and development

It was excessive stem elongation in infected rice plants that led to the discovery of gibberellins, and the more dramatic effects of gibberellins on higher plants. Unlike auxins, gibberellins promote elongation almost exclusively in intact plants rather than excised tissues. Nowhere is this more evident than in the control of internode elongation in genetic dwarfs. The relationship between dwarfing or internode-length genes and gibberellins was pioneered by the work of B. O. Phinney on maize (Zea mays) and P.W. Brian and coworkers on garden pea (Pisum sativum). Since these pioneering studies, experiments have been conducted with dwarf mutants of rice (Oryza sativa), bean (Phaseolus vulgaris), Arabidopsis thaliana, and several others. In all cases, application of exogenous gibberellin to the dwarf mutant restores a normal, tall phenotype (Fig.60). Exogenous gibberellin has no appreciable effect on the genetically normal plant.

### 13.2. ábra - Figure 60. The effect of gibberellic acid on dwarf pea seedlings

*Left: Control, showing reduced internode elongation characteristic of the dwarf growth habit. Right: Gibberellin treated with a 5 × 10⁻⁴ M foliar-drench of GA3. Note that gibberellin treatment increased stem elongation by simulating elongation of the internodes.*
Additional support for the role of gibberellins in stem elongation comes from the study of rosette plants. A rosette is essentially an extreme case of dwarfism in which the absence of any significant internode elongation results in a compact growth habit characterized by closely spaced leaves. The failure of internode to elongate may result from a genetic mutation, or may be environmentally induced. Regardless of the cause, hyper-elongation of stems in rosette plants is invariably brought about by the application of small amounts of gibberellin (Fig.61).

**13. ábra - Figure 61. Gibberellin-stimulated stem growth in a rosette genotype of Brassica napus. Treatments were (from left): 0, 0.5, 1.0, 10.0mg GA3 per plant, applied to the meristem.**

Environmentally limited rosette plants such as spinach (Spinacea oleraceae) and cabbage (Brassica sp.) generally do not flower in the rosette form. Just before flowering, these plants will undergo extensive internode elongation, a phenomenon known as bolting. Bolting is normally triggered by an environmental signal, either photoperiod (as in spinach) or a combination of low temperature and photoperiod (as in cabbage). We will return to the phenomena of photoperiod and cold requirement in later chapters. It is sufficient to note here that, under conditions normally conducive to the rosette habit, spinach, cabbage, and many other rosette plants can be induced to bolt by an exogenous application of gibberellic acid.

The above results suggest that (1) gibberellins are a limiting factor in the stem growth of rosette plants and (2) the effect of long days or cold treatment is to remove that limitation. These possibilities have been confirmed in spinach and Silene armeria, both photoperiodic plants requiring long days to flower, by the extensive investigations of J. A. D. Zeevaart and coworkers.

**13.3. 13.1.3 Gibberellins stimulate mobilization of nutrient reserves during germination of cereal grains**

Gibberellins initiate the mobilization of nutrient reserves stored in the endosperm, while auxins promote elongation of the embryonic axis. The auxins that support early embryo growth are largely derived from the breakdown of stored conjugates to free, active IAA while the gibberellins, at least in cereal grains, appear to be released by the hydrated embryo from a preformed GA pool. In Arabidopsis, on the other hand, seeds carrying mutations such as GA1, GA2, and GA3, that act early in gibberellin biosynthesis, fail to germinate but germination can be rescued by applying exogenous gibberellin.

A role for gibberellins in mobilization of reserves during seed germination was first suggested by experiments on germinating cereal grains in the late 1950s. Cereal grains such as rye, barley, and wheat have a protein-rich layer of cells called the aleurone which surrounds the starchy endosperm tissue. During germination, cells in the aleurone secrete a range of hydrolytic enzymes, including α-amylase and proteases, which are involved in the hydrolysis of carbohydrate and protein stored in the endosperm.

The involvement of gibberellins in enzyme secretion can be shown by a relatively simple experiment. Seeds of cereals such as barley are transected to produce two half-seeds (Fig.62).

**13.4. ábra - Figure 62. Gibberellin-stimulated secretion of α-amylase from barley half-seeds**
Embryo-less half-seeds were incubated on the surface of a starch-agar gel. After 48 hours, the gel was washed with iodine-potassium iodide (IKI), a reagent that reacts with starch to form a blue-black color. A clear circle, or halo, surrounding the half-seed indicates the digestion of starch by α-amylase. The control plate (left) contains four half-seeds but no added gibberellin. Two half-seeds produced no α-amylase while the other two exhibited low activity. The plate on the right contained 10 nanomoles gibberellic acid. Each of the gibberellin-treated half-seeds is surrounded by a large halo, indicating active α-amylase secretion.

One half-seed contains the embryo and the other half-seed does not. When imbibed, the embryo-containing half-seed will proceed to secrete α-amylase and other hydrolytic enzymes in order to digest the starchy endosperm, mobilize the resulting nutrients, and initiate germination. The half-seed without the embryo cannot, of course, germinate but neither does it produce elevated levels of α-amylase or any of the other hydrolytic enzymes required for germination. Treatment of the embryo-less half-seed with gibberellic acid, however, will stimulate the half-seed to produce high levels of α-amylase.

Experiments of this general nature have shown that the germinating embryo sends a signal, probably gibberellin, to the aleurone cells. There the gibberellin either activates or derepresses transcription of genes encoding the necessary hydrolytic enzymes. These enzymes are then released into the endosperm where they break down the starches and proteins to provide nutrients for the growing embryo (Fig.63).

13.5. ábra - Figure 63. A schematic illustrating gibberellin-induced release of enzymes and carbohydrate mobilization during germination of

2. 13.2 Cytokinins

2.1. 13.2.1 The discovery of cytokinins

The discovery of cytokinins came about because plant cells in culture would not divide. The first experimental evidence for chemical control of plant cell division was provided by Haberlandt in 1913, when he demonstrated
that phloem sap could cause nondividing, parenchymatous potato tuber tissue to revert to an actively dividing meristematic state. Other cell-division factors were later demonstrated in wounded bean pod tissue, extracts of Datura ovules, and the liquid (milky) endosperm of coconut.

In the 1940s and 1950s, plant tissue culture was attracting the attention of physiologists as a tool for study of cell division and development. One group, under the direction of F. Skoog at the University of Wisconsin, was studying the nutritional requirements of tissue cultures derived from tobacco stem segments. Skoog and coworkers found that stem tissue explants containing vascular tissue would proliferate on a defined medium containing auxin. On the same auxin-containing medium, however, tissue explants freed of vascular tissue would exhibit cell enlargement, but failed to divide. They soon found that extracts of vascular tissue, coconut milk, and yeast would all stimulate cell division in the presence of auxin.

C.O. Miller, then working as a postdoctoral student in Skoog’s laboratory, took on the task of isolating the active material. Miller was able to provisionally identify the active material as a adenine, one of the nitrogenous bases found in nucleic acids. This led to a search for active material in nucleic acid preparations, a source high in adenine. In a beautiful piece of serendipity, Miller sampled a bottle of herring sperm DNA which had been sitting on the laboratory shelf. The sample proved to be highly active, so a fresh supply of herring sperm DNA was ordered. Unfortunately, the fresh sample proved to be completely inactive. It turns out that the active principle in the original DNA sample had slowly accumulated as the DNA aged on the laboratory shelf. Activity could be generated in fresh DNA samples simply by artificially “aging” the sample with heat and acid.

In 1956, Miller and his colleagues reported the isolation and crystallization of a highly active substance, identified as the adenine derivative N6-furfurylaminopurine, from autoclaved herring sperm DNA. Because the compound elicited cell division, or cytokinesis, in tissue culture, Miller and his colleagues named the substance kinetin. In 1965, Skoog and his colleagues proposed the term cytokinin. Even though kinetin remains one of the most biologically active cytokinins, it is a synthetic derivative that has not yet been identified in plants. Curiously, however, there is a recent report that kinetin has been identified in human urine.

2.2. 13.2.2 Cytokinins are adenine derivatives

Naturally occurring cytokinins are all adenine derivatives with either an isoprene-related side chain or an aromatic (cyclic) side chain. The former are called isoprenoid cytokinins and the latter are called aromatic cytokinins. Although there is some variation depending on species and developmental stage, the most common isoprenoid cytokinins are N6-(2-isopentenyl)-adenine (iP), trans-zeatin (tZ), and dihydrozeatin (DZ) (Fig.64). The aromatic cytokinins, such as benzyladenine (BA) are less common and are found in only a few species.

The original cytokinin, kinetin, is a synthetic derivative that has not yet been identified in plants. Curiously, however, there is a recent report that kinetin has been identified in human urine.

13.6. ábra - Figure 64: The chemical structures of four representative cytokinins
Kinetin, the first compound found with cytokinin activity, is a synthetic cytokinin prepared by heating DNA. Isopen
tenyl adenine (iP) and trans-Zeatin, both isoprenoid-type cytokinins, are the most common naturally occurring cytokinins. Benzyladenine (BAP) is an aromatic cytokinin. The N6 position of adenine is indicated and the side chains are highlighted.

2.3. 13.2.3 Synthesis of cytokinins

A major site of cytokinin biosynthesis in higher plants is the root. High cytokinin levels have been found in roots, especially thermitotically active root tip, and in the xylem sap of roots from a variety of sources. It is generally concluded that roots are a principal source of cytokinins in most, if not all, plants and that they are transported to the aerial portion of the plant through the xylem. Indirect support for this conclusion is provided by the observation that excised leaves from many species can be maintained in a moist sand bed only if adventitious roots are permitted to form at the base of the petiole. If these roots do not form or are removed as they form, the leaves will quickly senesce. The delayed senescence when roots are allowed to form is apparently due to the presence of cytokinins, which are synthesized in the root and transported to the leaf through the vascular tissue.

Immature seeds and developing fruits also contain high levels of cytokinins; the first naturally occurring cytokinins were isolated from milky endosperm of maize and developing plum fruits. While there is some evidence that seeds and fruits are capable of synthesizing cytokinins, there is also evidence to the contrary. Thus, it remains equally possible that developing seeds, because of their high metabolic activity and rapid growth, may simply function as a sink for cytokinins transported from the roots. On the other hand, there is now
evidence that cytokinins are not always a long-distance messenger. As we will see later, meristematic cells in the shoot apical meristem and floral meristems in particular are under the control of locally produced cytokinins.

## 2.4. 13.2.4 Cytokinins delay senescence

At present, there are three lines of evidence indicating a role for cytokinins as inhibitors of senescence. First is the observation that exogenous application of cytokinin to detached leaves will delay the onset of senescence, maintain protein levels, and prevent chlorophyll breakdown. Application of cytokinins will also delay the natural senescence of leaves on intact plants. The second line of evidence consists of correlations between endogenous cytokinin content and senescence. For example, detached leaves that have been treated with auxin to induce root formation at the base of the petiole will remain healthy for weeks. In this case, the growing root is a site of cytokinin synthesis and the hormone is transported through the xylem to the leaf blade. If the roots are continually removed as they form, senescence of the leaf will be accelerated. It has also been observed that when a mature plant begins its natural senescence, there is a sharp decrease in the level of cytokinins exported from the root.

A third and particularly convincing line of evidence comes from recent studies employing recombinant DNA techniques. Tobacco plants (Nicotiana tobacum) have been transformed with the Agrobacterium gene for cytokinin biosynthesis, designated TMR (Fig. 65).

### 13.7. ábra - Figure 65. Tumor induction by Agrobacterium tumefaciens. (After Chilton 1983.)

13.8. ábra - Figure 66. Cytokinin control of senescence and bud growth in tobacco
Tobacco callus cells, genetically transformed such that cytokinin production could be stimulated by heat shock, were allowed to regenerate plantlets. (A) Transformed heat-shocked plantlets. (B) Untransformed heat-shocked plantlets. (C) Transformed controls (no heat shock). (D) Untransformed controls. Note especially the proliferation of lateral buds and absence of senescence in the transformed, heat-shocked plantlets. The large, white areas in B and D are senesced leaves. The transformed controls (C) do not, as expected, exhibit the cytokinin effect on lateral bud growth but do not exhibit senescence. This probably indicates the transformed gene is “leaky” and a small amount of cytokinin is produced in the absence of heat shock. (From Smart, C. et al. 1991. The Plant Cell 3:647. Copyright American Society of Plant Physiologists. Photo courtesy of C. Smart.)

The Agrobacterium TMR gene encodes for the enzyme isopentenyl transferase, which catalyzes the rate-limiting step in cytokinin biosynthesis. In this case, the TMR gene was unlinked to a heat shock promoter. A promoter is a sequence of DNA that signals where the transcription of messenger RNA (mRNA) should begin. The heat shock promoter is normally involved in the heat shock response of plants, which is induced by a brief period of high temperature. Normally, the heat shock response involves the synthesis of a new set of proteins called heat shock proteins. The heat shock promoter is thus active only when subjected to a high temperature treatment. By unlinking the TMR gene to the heat shock promoter, cytokinin biosynthesis can be turned on in the transformed plants simply by subjecting the plants to a brief period of high temperature. A heat shock of 42°C for 2 hours caused a 17-fold increase in zeatin levels in transformed plants compared with untransformed control plants. When subjected to heat shock on a weekly basis over a 12-week period, transformed plants exhibited a marked release of lateral buds from apical dominance as well as delayed senescence. Transformed but non-heat-shocked plants also remained green longer than normal plants but did not exhibit release from apical dominance. This is probably due to “leakiness” on the part of the promoter, allowing production of a very small but effective amount of cytokinin even at normal temperature. The mechanism by which cytokinins are able to delay senescence is not clear, but there is some evidence that cytokinins exert a role in mobilizing nutrients. The classic experiment of K. Mothes and coworkers was made a nutrient labeled with radioactive carbon (e.g., 14C-glycine) is applied to a leaf after a portion of the leaf has been treated with cytokinin. Invariably the radioactivity is transported to and accumulates in the region of cytokinin treatment. A variety of similar experiments has led to the hypothesis that cytokinins direct nutrient mobilization and retention by stimulating metabolism in the area of cytokinin application. This creates a new sink - an area that preferentially attracts metabolites from the region of application (the source). It is unlikely that cytokinins act directly through stimulating protein synthesis since the mobilization of nonmetabolites such as α-aminoisobutyric acid is directed by cytokinins equally well.

2.5. 13.2.5 Cytokinins have an important role in maintaining the shoot meristem

The fundamental characteristic of a plant meristem is the capacity to maintain the spatial distinction between dividing cells and differentiating cells. The entire course of plant development depends on maintaining that small population of perpetually dividing cells. Ever since Skoog and Miller demonstrated that cytokinins induced cell division and shoot regeneration fifty years ago, cytokinins have been routinely used to induce shoot formation in tissue culture for plant propagation and for the production of transgenic plants. It has always been assumed that cytokinins had a significant role in maintaining the meristem in planta, but direct evidence for such a role has been difficult to obtain. The traditional method involving exogenous application of cytokinins is something of a shotgun approach that provides relatively little solid information. When you simply spray a plant
with hormone solution, for example, it is virtually impossible to know how much hormone actually gets into the plant or where it goes. Moreover, excessive hormone levels may cause artifactual, nonphysiologic effects.

Several new lines of evidence, however, now point to a positive role for cytokinins in the shoot apical meristem. Most of these experiments involve reducing the in planta concentration of cytokinins, either by overexpressing appropriate genes in transgenic plants or through loss-of-function mutants. For example, cytokinin levels can be reduced in plant by overexpressing the genes for cytokinin oxidase/dehydrogenase (CKX), which degrades active cytokinins. Arabidopsis has seven CKX genes and, depending on which of these genes is over expressed, the cytokinin content can be reduced to 30 to 45 percent of wild type plants. The result in all cases was retarded shoot development; dwarfed, late flowering plants; and reduced size of the shoot meristem. The formation of leaf primordia was slower in cytokinin-deficient plants and the number of leaf cells was significantly reduced.

Where CKX was most strongly expressed, growth of the shoot was arrested completely shortly after germination. Similar results were obtained in experiments where the cytokinin content was reduced through loss-of-function mutants of the gene for IPT or of genes for known cytokinin receptors.

Further evidence for the maintenance of the shoot apical meristem by cytokinins is offered by the discovery of a cytokinin-deficient rice mutant that was given the intriguing name of lonely guy (log). Rice flowers are borne in a typical, highly branched grass inflorescence called a panicle. The flowers, or spikelets, normally contain a single pistil surrounded by several stamens. In log mutant plants, the size of the panicle was severely reduced. There were fewer branches and the branches bore abnormal flowers. Flowers were often reduced to no pistil and but a single stamen (hence the name lonely guy). Microscopic studies revealed that after the transition from vegetative to reproductive stage the normally dome-shaped floral meristem flattens and the differentiation of floral organs is prematurely shut down.

Clearly, the log mutant is characterized by a deficient meristem, but why? When the LOG gene was isolated and cloned, it was found that expression of the LOG gene is localized in regions of active cell division in the meristem such as the apex of the meristem and branch primordia. It was also found that LOG encodes a phosphoribohydrolase enzyme. This means that the enzyme activates cytokinins by removing the ribose phosphate group from an inactive cytokinin nucleotide to leave the active free base. The absence of this enzyme in the log mutant thus reduces the level of active cytokinin in the critical region of cell division and the result is an improperly maintained meristem.

### 3. 13.3 Abscisic acid

Unlike auxins, gibberellins, and cytokinins, the hormone abscisic acid (ABA) is represented by a single 15-carbon sesquiterpene (Fig.67).

**13.9. ábra - Figure 67. Abscisic acid is a class of hormones represented by a single compound**

ABA also appears to have a more limited range of specific effects than auxins, gibberellins, and cytokinins. The name is based on the once held belief that it was involved in the abscission of leaves and other organs. It now appears to have nothing to do with abscission, but the name has stuck. The primary functions of ABA are (1) prohibiting precocious germination and promoting dormancy in seeds and (2) inducing stomatal closure and the production of molecules that protect cells against desiccation in times of water stress. ABA has also been implicated in other developmental responses, including the induction of storage protein synthesis in seeds, heterophyllly (leaves of different shape on the same plant), initiation of secondary roots, flowering, and senescence.

### 3.1. 13.3.1 Discovery of ABA
As more investigators became interested in plant hormone research, it soon became evident that ether extracts of plant material - used to extract auxins - frequently contained substances that interfered with the auxin response in the Avena coleoptile curvature test. Initially, the principal interest of investigators was to rid extracts of these interfering substances. As time went on, however, interest turned toward the possibility that these inhibitors might themselves be growth regulators in their own right. The advent of paper chromatography as an analytical tool made it possible to achieve better separation of the various substances in crude extracts. In 1953, Bennet-Clark and Kefferd reported that plant extracts contained, in addition to IAA, a substance that inhibited growth of coleoptile sections, which they called inhibitor β. The observation that large amounts of inhibitor β could be isolated from axillary buds and the outer layer of dormant potato tuber led Kefferd to suggest that it was involved in apical dominance and maintaining dormancy in potatoes. Meanwhile, other investigators reported the occurrence of inhibitors in buds and leaves that appeared to correlate with the onset of dormancy in woody plants.

In 1964, P. F. Waring proposed the term “dormin” for these endogenous, dormancy-inducing substances. In another line of study, substances that accelerated abscission were isolated from senescing leaves of bean and from cotton and lupin fruits. These substances would accelerate abscission when applied to excised abscission zones and were called “abscission II.” These several lines of study came to a head in the mid-1960s when three laboratories independently reported the purification and chemical characterization of abscisin II, inhibitor β, and dormin. All three substances proved to be chemically identical.

It is not unusual in such cases that there was some disagreement over what this substance should be called. Although abscisin II had priority (it was the first to be crystallized and chemically characterized), some felt the term awkward and argued it did not adequately describe its range of effects. Finally, a panel of scientists active in research on abscisin II and dormin was charged with proposing an acceptable name. The name abscisic acid and abbreviation ABA were recommended by this panel to the 1967 International Conference on Plant Growth Substances, which met in Ottawa. The recommendation was accepted by the Conference and the term abscisic acid is now in universal use.

### 3.2. 13.3.2 Synthesis of ABA

Once the structure of ABA had been determined, two possible pathways for the synthesis of ABA were proposed. In the “direct pathway,” ABA would be synthesized from a 15-carbon terpenoid precursor such as farnesyl diphosphate.

By the late 1970s it had been clearly established that this pathway was operative in certain fungal plant pathogens that actively synthesized ABA, but not in plants themselves. According to the second, or “indirect pathway,” ABA as β-carotene. Originally proposed in the late 1960s, the indirect pathway was based on structural similarities between carotenoid pigments and ABA and has since received support from a variety of biochemical studies, 18O2-labeling experiments, and, most recently, the characterization of ABA biosynthetic mutants. The cleavage of carotenoids, especially β-carotene, to produce useful biochemicals is not without precedent. The cyanobacterium Microcystis, for example, produces a C10 metabolite by cleavage of β-carotene. Mammals produce vitamin A by cleavage of β-carotene and cleavage of β-carotene to produce 2 molecules of the photoreceptor retinal (C20) has been reported. There is now a growing body of evidence supporting the indirect synthesis of ABA from β-carotene via the 40-carbon terpene violaxanthin (Fig.68).

13.10. ábra - Figure 68. A flow sheet for the biosynthesis of abscisic acid
ABA biosynthesis begins in the chloroplast with the synthesis of isopentenylpyrophosphate (IPP) from glyceraldehydes-3-phosphate (G3P) and pyruvate via the methylerthritol-4-phosphate (MEP) pathway. IPP in the chloroplast gives rise to a variety of C10, C20, and C40 terpenoids, including β-carotene. β-Carotene is converted to violaxanthin, which is cleaved by the enzyme nine-cis-epoxycarotenoid dioxygenase (NCED) to yield xanthoxin, a C15 precursor to ABA, and a 25-carbon “by-product.”

First, a series of viviparous mutants in maize (described further below) were found to have reduced levels of both carotenoids and ABA. These mutants, shown to be affected in the early steps of carotenoid biosynthesis, establish a strong correlation between carotenoid and ABA biosynthesis. Second, the carbon skeleton of ABA and the position of the oxygen-containing substituents are very similar to that of violaxanthin. J. A. D. Zeevaart and his colleagues compared the incorporation of 18O2, a stable isotope of oxygen, into ABA in water-stressed leaves and turgid leaves of several species. The pattern of 18O2-enrichment in the carboxyl group of ABA was consistent with the cleavage of a xanthophyll and its rapid conversion to ABA in water-stressed leaves. Third, it is known that violaxanthin can be degraded in the light in vitro to a 15-carbon derivative, xanthoxin, a natural constituent of plants. If radio-labeled xanthoxin is fed to bean or tomato plants, some of the radioactivity appears in ABA. In ABA-deficient tomato mutants, however, conversion of radio-labeled xanthoxin into ABA is reduced relative to wildtype plants. Finally, at least two groups have reported a stoichiometric relationship between losses of violaxanthin and increases in ABA in stressed etiolated bean leaves. Although ABA is synthesized in the cytosol, its biosynthetic pathway begins in the chloroplast (and possibly other plastids in nongreen cells), which is where carotenoid pigments are produced. The critical enzyme is nine-cis-epoxycarotenoid dioxygenase (NCED). This enzyme cleaves the 40-carbon carotenoid violaxanthin to produce a 15-carbon product, xanthoxin, and a 25-carbon “by-product.” Xanthoxin is then converted to abscisic aldehyde by an alcohol dehydrogenase. Abscisic aldehyde is in turn oxidized to abscisic acid by abscisic aldehyde oxidase. The enzyme NCED and, consequently xanthoxin production, is known to be targeted in the chloroplast while the alcohol dehydrogenase and abscisic aldehyde oxidase are located in the cytosol. This means that xanthoxin must migrate from the chloroplast into the cytosol, although the mechanism of migration is not yet known.

3.3. 13.3.3 Abscisic acid regulates embryo maturation and seed germination

The development of embryos and subsequent germination of the seed is characterized by often dramatic changes in hormone levels (Fig.69).

13.11. ábra - Figure 69. The activity of hormones during seed development and germination
Cytokinin (CK) are present during the early stages of development, when cell division is most active. The concentration of cytokinins then declines while the concentrations of gibberellin (GA) and auxin (IAA) increase to support active cell enlargement. Abscisic acid (ABA) concentration increases in the later stages to prevent precocious germination. After a quiescent period, the release of gibberellins activates nutrient mobilization and IAA levels increase to stimulate cellular enlargement in the young seedling.

In most seeds, cytokinin levels are highest during the very early stages of embryo development when the rate of cell division is also highest. As the cytokinin level declines and the seed enters a period of rapid cell enlargement, both GA and IAA levels increase. In the early stages of embryogenesis, there is little or no detectable ABA. It is only during the latter stages of embryo development, as GA and IAA levels begin to decline, that ABA levels begin to rise. ABA levels generally peak during the maturation stage, when seed volume and dry weight also reach a maximum, and then return to lower levels in the dry seed. Maturation of the embryo is characterized by cessation of embryo growth, accumulation of nutrient reserves in the endosperm, and the development of tolerance to desiccation. The timing of ABA accumulation to coincide with embryo maturation reflects the critical role that ABA plays in the maturation process. One of the functions of a seed, of course, is to disperse the population and ensure survival of the species through unfavorable conditions. A seed would be of little value if the embryo did not enter dormancy but continued to grow and establish a new plant before dispersal could occur. One function of ABA is to prevent such precocious germination, or vivipary, while the seed is still on the mother plant. The relationship between ABA and precocious germination is clear. Vivipary can be chemically induced in maize by treatment of the developing ear at the appropriate time with fluridone, a chemical inhibitor of carotenoid biosynthesis. Since carotenoids and ABA share early biosynthetic steps, fluridone inhibits biosynthesis of ABA as well. Fluridone-induced vivipary can be at least partially alleviated by application of exogenous ABA. Soybean embryos can be encouraged to germinate precociously by treatments such as washing or slow drying, both of which lower the endogenous ABA level. Precocious germination will occur when the ABA concentration is reduced to 3 to 4 g per g fresh weight of seed, a level that is not normally reached until the late stages of seed maturation. The strongest indication of a role for ABA in preventing precocious germination, however, comes from the study of viviparous mutants. At least four viviparous mutants in maize (vp2, vp5, vp7, vp9) are known to be ABA-biosynthetic mutants with reduced levels of ABA in the seeds. One maize mutant, vp1, appears to have normal ABA levels but is missing what is believed to be an ABA-specific transcription factor. All of these mutants germinate prematurely on the cob before the seeds have entered dormancy. Viviparous mutants are also known for Arabidopsis. ABA also stimulates protein accumulation in the latter stages of soybean embryo development and is known to prevent GA-induced α-amylase biosynthesis in cereal grains. All of these results establish a strong connection between ABA and seed maturation and/or prevention of precocious germination. ABA also initiates desiccation of the seed, although the mechanisms are unknown. This may involve ABA regulation of genes, which encode proteins that are involved in desiccation tolerance.

**3.4. 13.3.4 Abscisic acid mediates response to water stress**

Plants generally respond to acute water deficits by closing their stomata in order to match transpirational water loss from the leaf surface with the rate at which water can be resupplied by the roots. Since the discovery of ABA in the late 1960s, it has been known to have a prominent role in stomatal closure during water stress. In fact, ABA has long been recognized as antitranspirant because of its capacity to induce stomatal closure and thus reduce water loss through transpiration. ABA accumulates in water-stressed (that is, wilted) leaves and exogenous application of ABA is a powerful inhibitor of stomatal opening. Furthermore, two tomato mutants, known as flacca and sitiens, fail to accumulate normal levels of ABA and both wilt very readily. The precise role of ABA in stomatal closure in water-stressed with certainty. This is because ABA is ubiquitous, often occurring in high concentrations in nonstressed tissue. In addition, some early studies indicated that stomata
would begin to close before increases in ABA content could be detected. According to current thinking, the initial detection of water stress in leaves is related to its effects on photosynthesis. Inhibition of electron transport and photophosphorylation in the chloroplasts would disrupt proton accumulation in the thylakoid lumen and lower the stroma pH. At the same time, there is an increase in the pH of the apoplast surrounding the mesophyll cells. The resulting pH gradient stimulates a release of ABA from the mesophyll cells into the apoplast, where it can be carried in the transpiration stream to the guard cells (Fig. 70).

13.12. ábra - Figure 70. ABA movements in the apoplast

ABA synthesized in the roots is carried to the leaf mesophyll cells (heavy arrows) in the transpiration stream (light arrows). ABA equilibrates with the chloroplasts of the photosynthetic mesophyll cells or is carried to the stomatal guard cells in the apoplast.

As noted above, wilted leaves accumulate large quantities of ABA. In most cases, however, stomatal closure begins before there is any significant increase in the ABA concentration. This could be explained by the release of stored ABA into the apoplast, which occurs early enough and in sufficient quantity - the apoplast concentration will at least double - to account for initial closure. Increased ABA synthesis follows and serves to prolong the closing effect.

Stomatal closure does not always rely on the perception of water deficits and signals arising within the leaves. In some cases it appears that the stomata close in response to soil desiccation well before there is any measurable reduction of turgor in the leaf mesophyll cells. Several studies have indicated a feed-forward control system that originates in the roots and transmits information to the stomata. In these experiments, water deficits can then be introduced by withholding water from one container while the other is watered regularly. Control plants receive regular watering of both containers. Stomatal opening along with factors such as ABA levels, water potential, and turgor are compared between half-watered plants and fully watered controls. Typically, stomatal conductance, a measure of stomatal opening, declines within a few days of withholding water from the roots, yet there is no measurable change in water potential or loss of turgor in the leaves. In experiments with day flower (Commelina communis), there was a significant increase in ABA content of the roots in the dry container and in the leaf epidermis. Furthermore, ABA is readily translocated from roots to the leaves in the transpiration stream, even when roots are exposed to dry air. These results suggest that ABA is involved in some kind of early warning system that communicates information about soil water potential to the leaves.

4. 13.4 Ethylene

Ethylene is another class of hormones with a single representative. Ethylene is a simple gaseous hydrocarbon with the chemical structure H2C=CH2. Ethylene is apparently not required for normal vegetative growth, although it can have a significant impact on the development of roots and shoots. Ethylene appears to be synthesized primarily in response to stress and may be produced in large amounts by tissues undergoing senescence or ripening. Ethylene is commonly used to enhance ripening in bananas and other fruits that are picked green for shipment. Ethylene is frequently produced when high concentrations of auxins are supplied to plant tissues and many of the inhibitory responses to exogenously applied auxin appear to be due to auxin-stimulated ethylene release rather than auxin itself.
Ethylene occurs in all plant organs - roots, stems, leaves, bulbs, tubers, fruits, seeds, and so on - although the rate of production may vary depending on the stage of development. Ethylene production will also vary from tissue to tissue within the organ, but is frequently located in peripheral tissues. In peach and avocado seeds, for example, ethylene production appears to be localized primarily in the seed coats, while in tomato fruit and mung bean hypocotyls it originates from the epidermal regions. The off-gassing of ethylene by natural vegetation is also a significant source of atmospheric volatile organic carbon (VOC).

4.1. 13.4.1 The discovery of ethylene

The effect of ethylene on plants was originally described by Dimitry Nikolayevich Neljubow, a graduate student in Russia in 1886, who found that abnormal growth of dark-grown pea seedlings could be traced to ethylene emanating from illuminating gas. Interest in ethylene as a plant growth factor, however, did not gain real momentum until it was found to have commercial implications.

Those whose business involves the shipping and storing of fruit have long been aware that ripe and rotting fruit could accelerate the ripening of other fruit stored nearby. For example, bananas picked in Cuba and shipped by boat often arrived in New York in an overripe and unmarketable condition. One of the earliest reports that these effects were due to a volatile substance given off by plant tissue was published in 1910 by H. H. Cousins in an annual report of the Jamaican Department of Agriculture. He discovered that ripe oranges released a volatile product that would accelerate ripening of bananas stored with them. A number of similar reports appeared in the early 1930s, showing that volatile emanations from apples caused epinasty in tomato seedlings and respiratory changes associated with the ripening process. In 1934, R. Gane provided indisputable evidence that the volatile substance was ethylene.

4.2. 13.4.2 Synthesis of ethylene

Despite the early discovery of ethylene, its known importance in plant development, and its relatively uncomplicated chemistry, the pathway for ethylene biosynthesis initially proved difficult to unravel. This is partly because there were a large number of potential precursors (sugars, organic acids, or peptides) known to be present in plant tissues. In addition, until recently, the enzymes involved have proven too labile to isolate and study in vitro. Consequently, most of the work has been carried out in vivo, with all the pitfalls inherent in such experiments. Moreover, ethylene is a volatile gas and available analytical methods for its measurement were simply too insensitive. It wasn’t until the early 1960s that developments in gas chromatography made it possible to analyze ethylene at physiologically active concentrations. With the advent of gas chromatography, the study of ethylene began to advance rapidly. M. Lieberman and L. W. Mapson first demonstrated in 1964 that methionine was rapidly converted to ethylene in a cell-free, nonenzymatic model system. In subsequent studies, Lieberman and coworkers confirmed that plant tissues such as apple fruit converted [14C]-methionine to [14C]-ethylene and that the ethylene was derived from the third and fourth carbons of methionine. Little progress was made until 1977 when D. Adams and F. Yang demonstrated that S-adenosylmethionine (SAM) was an intermediate in the conversion of methionine to ethylene by apple tissue. In 1979, Adams and Yang further demonstrated the accumulation of 1-aminocyclopropane-1-carboxylic acid (ACC) in apple tissue fed [13C]-methionine under anaerobic conditions - conditions that inhibit the production of ethylene. However, upon reintroduction of oxygen, the labeled ACC was rapidly converted to ethylene. ACC is a nonprotein amino acid that had been isolated from ripe apples in 1957, but its relationship to ethylene was not obvious at that time. These results established that ACC is an intermediate in the biosynthesis of ethylene. The three-step pathway for ethylene biosynthesis in higher plants is shown in Figure X. In the first step, an adenosine group (i.e., adenine plus ribose) is donated to methionine by a molecule of ATP, thus forming SAM. An ATP requirement is consistent with earlier evidence that ethylene production is blocked by inhibitors of oxidative phosphorylation, such as 2,4-dinitrophenol.

Conversion of methionine to SAM is catalyzed by the enzyme methionine adenosyltransferase or SAM synthetase.

The cleavage of SAM to yield 5-methylthioadenosine (MTA) and ACC, mediated by the enzyme ACC synthase, is the rate-limiting step. ACC synthase was the first enzyme in the pathway to be studied in detail. The enzyme has been partially purified from tomato and apple fruit but, because of its instability and low abundance, progress toward its purification and characterization has been slow. More recently, genes for ACC synthase have been isolated from zucchinis (Cucurbita) fruit and tomato pericarp tissue. The cloned genes direct the synthesis of active ACC synthase in the bacterium E. coli and yeast, making it possible to produce the enzyme in sufficient quantity for further study. The enzyme that catalyzes the oxidation of ACC to ethylene, previously
referred to as the ethylene-forming enzyme but now known as ACC oxidase, proved especially difficult to isolate in the active form. ACC oxidase was finally identified when a gene cloned from ripening tomato fruit (pTOM13) was unlinked to ethylene production.

Another important aspect of ethylene biosynthesis is the limited amount of free methionine available in plants. In order to sustain normal rates of ethylene production, the sulfur released during ethylene formation must be recycled back to methionine. This is accomplished by what is commonly referred to as the methionine cycle (Fig.71).

13.13. ábra - Figure 71. A scheme for ethylene biosynthesis in higher plants

![Diagram of ethylene biosynthesis](image)

*The enzymes are I: SAM synthetase; II: ACC synthase; and III: ACC oxidase. The ethylene group is highlighted in yellow. The Yang cycle for sulfur recovery is highlighted in orange.*

This cycle is also known as the Yang cycle, after S. F. Yang, who carried out much of the pioneering work on ethylene biosynthesis. Double-labeling experiments have shown that the CH3S-group is salvaged and recycled as a unit. The remaining four carbon atoms of methionine are supplied by the ribose moiety of the ATP used originally to form SAM. The amino group is provided by a transamination reaction.

Ethylene production is promoted by a number of factors including IAA, wounding, and water stress, principally by the induction of the synthesis of ACC synthase. Induction of this enzyme in plant tissues is blocked by inhibitors of both protein and RNA synthesis, suggesting that induction probably occurs at the transcriptional level. In E. coli carrying the cloned ACC synthase gene, the physical abundance of ACC synthase messenger RNA also increases in response to IAA and wounding. Control of ethylene production thus appears to be exercised primarily through transcriptional regulation of the ACC synthase gene. Ethylene production is also stimulated by ethylene itself, a form of autocatalysis. This is commonly seen in ripening fruits where ethylene apparently stimulates an increase in both ACC synthesis and its subsequent conversion to ethylene.

4.3. 13.4.4 Climacteric fruit

Ethylene is known primarily for its promotion of fruit ripening and senescence. Ethylene control of fruit development has been studied extensively in tomato, which is a climacteric fruit. During the development of climacteric fruits there is a characteristic developmentally regulated burst in respiration called the climacteric rise. The climacteric rise is normally accompanied by ethylene production and is followed by the expression of a set of genes that enhance ripening-related activities such as development of fruit color, flavor, and texture.

The tomato never ripe mutant is insensitive to ethylene because it has a defective ethylene receptor protein. Consequently, the “ripening genes” are not expressed and, although the fruit reaches full size, it never develops the red color, flavor, and texture characteristic of a ripe tomato.

Ethylene has been shown to stimulate elongation of stems, petioles, roots, and floral structures of aquatic and semiaquatic plants. The effect is particularly noted in aquatic plants because submergence reduces gas dispersion and thus maintains higher internal ethylene levels. In rice, ethylene is ineffective in the presence of
saturating levels of gibberellins, which also promotes stem elongation. Moreover, ethylene promotes gibberellin synthesis in rice and the elongation effect can be blocked with antigibberellins, which suggests that gibberellin mediates this ethylene effect. By contrast, root and shoot elongation in nonaquatic plants such as peas (Pisum sativum) is inhibited by ethylene. Ethylene stimulates many inhibitory and abnormal growth responses such as the swelling of stem tissues and the downward curvature of leaves, or epinasty. Leaf epinasty occurs because of excessive cell elongation on the adaxial (i.e., upper) side of the petiole. Epinasty is a common response to water logging of flood-sensitive plants such as tomato (Lycopersicum) and is actually a response to anoxia in the region of the roots. The more vertical orientation of epinastic leaves reduces the absorption of solar energy and, consequently, transpirational water loss. This helps to bring water loss more into line with reduced capacity for water uptake in plants suffering root anoxia. A role for ethylene has also been noted for promotion of seed germination, inhibition of bud break, reduced apical dominance, fruit ripening, cell death, and pathogen responses. Ethylene can be a problem in commercial greenhouse that are heated with gas-fired heating systems.

5. 13.5 Literature


6. 13.6 Questions

1. What is the significance of the ‘‘G’’ number assigned to each gibberellin?

2. Describe hormonal involvement in nutrient mobilization in germinating cereal grains!

3. What is the apparent role of gibberellins in the shoot apical meristem?

4. What is the evidence that ABA mediates responses to water stress?

5. What is the - ‘‘climacteric’’-? Give some samples for climacteric fruits!
14. fejezet - 14. Physiology of flowering

Plants, to begin with go through a period of vegetative growth. The extent of vegetative growth is endowed with its genetic potentiality. Accordingly, they may grow into herbs or shrubs and some may develop into trees or climbers. Generally, every plant after going through a period of vegetative growth, responding to environmental clues, start producing floral structures, which may be in the form of characteristic single flowers or inflorescences.

Many plants for that matter, a large number of plant species (higher plants), after a period of vegetative growth, start flowering irrespective of the season. But some plants flower only in a particular season of the year. Based on the duration required for the plants to produce flowers, they have been classified into annuals, biennials and perennials. All plants have to acquire ripeness to flowering. Annuals complete their vegetative growth and flowering in one season and then they die. Biennials produce vegetative growth in one season and flower in the next season. But perennials remain for many years and flower seasonally.

In fact, some trees do not flower till they reach a certain age. For example, coconut and areca nut plants start producing flowers only when they reach an age of 6-8 years. On the other hand in the case of bamboo plants, they grow for a number of years, and flower only once in their life span. As soon as they flower, produce seeds and plants die (monocarpic plants). Interestingly there are many plants, which flower throughout the year, ex., Catharathus roseus.

Aestivation of some flowers- refers to the organization of each floral part with respect to each other, especially holds good for sepal and petals.

Plants growing in different regions of the globe are exposed to different climatic conditions and different day lengths. In fact they are adapted to such environs in such a way, they exhibit alternate vegetative and flowering cycles. It means that plants with their inherent genetic potentiality interact with environmental conditions; accordingly, they respond and behave. Humans, homo sapiens present day species, just about 25000yrs to 40000yrs old, copulated with Homo eructus, mostly in Asia and made them extinct; when they evolved and colonized sites of their own, after observing for many centuries the above said natural phenomenon, they have devised different methods to cultivate crop plants in different seasons of the year, so as to get the harvest at the right time of the year. They also domesticated animals for their use.

The common knowledge of the farmer has been extended and explained by plant physiologists; why and how the said plants behave in response to different environmental conditions.

Plants have all the needed signal transduction pathways to respond to environmental signals. Such signal pathways has been worked out in Arabidopsis.

1. 14.1 Discovery of flowering response

Though it is a common knowledge that different kinds of plants respond to different seasons of the year in producing the flower, it was left to G.Gassner & W.W. Garner to explain the phenomenon by their pioneering scientific studies. Gassner observed that winter variety of petkus rye plants called Secale cereal, responded favorably to cold treatments. Almost at the same period of time, Garner and Allard demonstrated how plants produce flower in response to different lengths of the day and night in a 24 hours day cycle. The above two phenomenon are popularly called as Vernalization and Photoperiodism respectively. The above studies have lead to the discovery of how plants rhythmically respond and behave to day and night duration or to temperature fluctuation in different seasons of the year and they also observed rhythmical behavior of the plants which is referred to as ‘biological rhythm’. And the operational time measuring system found with in the plant structures is called ‘Biological Clock’.

2. 14.2 Photoperiodism (PP)

Earth, because of its revolution on its own axis and rotation around the sun, exhibits a period of day and night and seasonal changes. The duration of the day and night again shows variations because of the angle and
distance between the earth and the sun at any given time of the year. Thus, plants and animals living on different parts of latitudes or longitudes are subjected to different periods of photo periods and different temperatures at different seasons of the year.

If we use three points or places on the globe, located at different positions as the reference point, to measure the day and night periods, it will be apparent how different are the day periods and temperatures of such places. Brazil in South America and Congo in Africa exhibit almost 12 hours of day and 12 hours of night in all the months of a year. But a city like Philadelphia located in the east coast of USA at latitude of 40 degree N, in the month of December; it experiences 9 hours of day and 15 hours of night. On the other hand, in the month of June, the day period is 14 ½ hours and night is 9 ½ hours long. Similarly, cities of Norway, during December, experience 6 hours of day and 18 hours of night, but in June, it enjoys 18 hours of day and 6 hours of night. Such day periods also accompany with changes in extreme temperatures. The above observations suggest that organisms living in these regions are subjected to seasonal variations of day and night and also to changes in seasonal temperature fluctuations.

Garner and Allard, while working the department of Agricultural Station, Beltsville, Maryland, USA, demonstrated remarkable relationship between the effect of the day period and flowering in a mutant tobacco plant called Maryland Mammoth. They observed that the mutant failed to produce flowers but tall, so they are called Maryland Mammoth. They also observed that the same plant started flowering in summer under field conditions. But the same plant started flowering when transferred to green house where it was subjected to short day and long night conditions. So the plant was called short day plant. Since then, a large number of plants have been subjected to various cycles of photoperiod i.e. treatment and according to their responses, plants have been classified into different groups. The flowering response in plants to photoperiodic treatment is now called photoperiodism. Light induced responses in photo morphogenesis are many and intricate, and this can be only represented in the form of network.

Another important factor that affects the floral induction is the intensity of light. If the light provided is of low intensity i.e., less than 100 ft candles, flower production is totally inhibited, though the meristems are organized into floral primordia. But if the intensity of light is increased above a critical level, the number of flowers produced also increases up to certain level. This is because the light intensity affects the total yield of photosynthate, so flower production is also affected by the said factor.

Finer analysis of the active part of white light that is effective is photoperiodic inductions reveals that the red light at 660 mm and far-red light at 730 mm are the most effective wavelengths in inducing or inhibiting the flower initiation. It has been established that continuous far-red irradiation inhibits flowering in long day plants, on the contrary, continuous red light treatment blocks flowering in short day plants.

The dramatic effects of red light and far red light can be demonstrated on a short day plant like xanthium. It is known that a short break in continuous dark period with white light in a short day plant brings about the total inhibition of flowering. If the short break is due to red light, the inhibition is 100%, but if the break is due to far red light flowering is not inhibited. Interestingly, if the red light and far red treatment is repeated alternately but ending in Far Red as short breaks results in the reduction of total number of flowers produced. If the number of cycles is extended the flowering will be totally inhibited though the last light treatment is far red light. This is possibly due to the breakdown or exhaustion of some intrinsic factors generated during short day treatments.

14.1. ábra - Figure 72.
Arabidopsis thaliana flowers rapidly under long days but not under short days. Plants on the left were grown under long days of 16 hours light, and are flowering and producing seeds. Plants on the right are the same age, but were grown under short days of 10 hours light, and are not flowering.

3. 14.3 Phytochrome as the Photoreceptor

The effectiveness of red light and far red light inducing or inhibiting the induction of flowers strongly suggests the presence of some substance or substances that could absorb light in the said wavelengths. By absorbing the light at particular wavelength, the said substances probably undergo excitation of chromophore and the protein bound undergoes conformational change.

This protein complex induces signal transducing activities leading to flower induction. Search for such a compound in plants resulted in the discovery of a blue/yellow colored pigment called phytochrome.

This complex when get excited with the absorption of light at red wave length, its protein gets activated and acts as an aspartate kinase and it autophosphorylates itself.

When phytochromes were discovered there was great excitement among plant physiologists. This led to intensive research work on various aspects of the structure and functions of phytochromes in plant morphogenesis. Now scientists have extracted and identified five phytochromes; Phy A to Phy E. Yet in recent years another pigment was discovered, called Cryptochrome whose role is not well discerned.

14.2. ábra - Figure 73. The activated form inactivated from a chromophore

The pure form of phytochrome appears blue/yellow colored pigment in solution form. Phytochrome, in fact, is made up of two moieties; one is a protein and the other is a chromophoric component. The phytochrome-associated protein has been isolated from maize seedlings and other sources. The Mole. wt. of phytochrome is 123-125 KD and it is a tetramer. But chromophore part is made up of linear chain of four pyrole rings. The protein subunits are firmly bound to the A-pyrole ring via S-bond of the chromophore unit (chromophorobilin). With the absorption of light at 660 nm or 730 nm, the double bonds found within the chromophore get disturbed and shifted. These changes in turn bring about conformational changes in 3-D structure of the pigment and also in protein chains either in in trans form (activated) or cis form (inactive form) (Fig.73). Probably, the above said changes due to absorption of light transform them into excited form of molecules and they intum elicit certain physiological functions in the cells.

In the earlier days of its discovery, people suspected the presence of two kinds of phytochrome pigments because they showed different absorption spectrum at 660 nm and 730 nm. But Norris and others, using dual wavelength spectrophotometer, demonstrated that the same phytochrome pigment exists in two alternate forms. They are called red light absorbing pigment and far red light absorbing pigment. The Pr form after absorbing red light gets transformed into Pfr form which by absorbing Far red light gets converted back to PR form. The Pfr form naturally undergoes decay back to Pr form. Thus phytochrome exhibit dual forms. The concentration of each form is dependent upon quality of the light source and the physiological state of the cell.

The PFR form of the pigment formed due to the absorption of red light is not very stable. It decays back to PR form or it is destroyed by some enzymes in dark condition, but this process is slow. On the other hand, if the PFR form absorbs far red light, it gets converted to PR quickly. But the decay of PFR pigment to PR form on its own takes place in dark or it is converted by certain enzymes and it is temperature dependent. In the presence of oxygen, the pigment undergoes irreversible destruction. In spite of their labileness and sensitivity, they remain quite stable at pH 6 and pH 8. Furthermore, the stability of these forms of pigments is controlled by the firm binding of protein moiety to the chromophore part of the pigment.
Activated phytochrome undergoes autophosphorylation at serine/threonine residue, which then binds to its receptor protein and moves into the nucleus, where it associates with transcriptional factors and activates their target genes whose products in turn activate other genes and promote flowering.

### 4. 14.4 Role of phytochromes in flower induction

Phytochromes being omnipresent in the plant body, they are always subjected to both red and far red radiations in the day conditions. Accumulation of Pr forms and Pfr forms of phytochrome in sufficient amounts in plants is critical and important. The effective concentration of any of these forms over a threshold values in the perceptive organs like leaves is essential to bring about certain biochemical functions, which may ultimately lead to the induction of flowers.

In long day plants, the Pr form of the pigment by absorbing red light throughout the day transforms the substance to Pfr form and it accumulates in greater amounts. Such Pfr pigments, when preset in higher concentration above the threshold value, activate the cell machinery and ultimately induces flowering. However, recent studies indicate that the PIR form alone is not active, but it also requires another substance called X whose properties are not well characterized. The PER-X complex is believed to be highly effective in inducing flower formation in long day plants. The X factor is known to be Phytochrome interacting factor (PIF3).

Light activated Phy-A binds to the receptor FHY1-FHL (FHY means elongated hypocotyls) that enters the nucleus where it activates light response genes including flowering genes. Accumulation PhyA represses the production of FHY3-FAR1 for they bind to gene loci of the same. With the dissociation of Phy-A from FHY1-FHL the genes for FHY3 and PAR1 get activated to produce FHY1-FHL that is found in cytosol after transcription and translation, which are used for the binding of Activated Phy-A.

On the contrary in short day plants, because of long dark periods, whatever Pfr pigments formed in the day conditions are subjected to decay back to Pr form. However, higher levels of Pr pigments are effective in inducing flower formation in short day plants. Conversely, higher amounts of Pr forms inhibit flower initiation in long day plants and PIR forms prevent initiation of flowers in short day plants. So the kind of pigment or the form of pigment that has a promotive effect on one kind of plant acts as an inhibitor to the other kind. The dual form of pigments performing dual role is intriguing.

### 5. 14.5 Effect of GA on Flowering

Fascinating aspect of flower inducing substance(s) is that when the extracts, obtained from photo induced leaves of Xanthium is applied to lemma plant kept under non inductive conditions, the extracts induce flowering in lemma plants. However, the induction of flowering by the extracts should be supplemented with gibberellins, without which the extracts alone or gibberellins alone has no effect. This experiment suggests that gibberellins are probably one of the components of elusive florigin (like elusive “Himalayan snow man”), but the nature of the other component is still a mystery.

It is very well known that gibberellins induces bolting and flowering in rosette leaved long day plants, but not in short day plants (with some exceptions). In long day plants, GA not only stimulates the elongation the condensed internodes, but at the same time, it also promotes the formation of factors needed for flowering. Thus GA treatment substitutes photoperiodic treatment in long day plants. Added to this complexity, application of high concentrations of gibberellins and cytokinins to the callus, obtained from Arachis hypogeae (peanut plant), results in the induction of flowers directly from the callus. Paradoxically ABA, a growth inhibitor, is very effective in inducing flowers in some short day plants like Fragilis, Pharbatis, etc. But ABA does not induced flowers in xanthium, another short day plant. This particular case is very interesting, at the same time, it is also intriguing and it further raises doubts about the existence of florigin per se. But such experimental results are very few and conditions used for such materials have to be carefully analyzed. Much more perplexing that is observed in some cases is that the application of cytokinins to the whole plant induces flowering, when the plant is at a particular stage of development (Fig. 74.).

Based on gibberellin’s promotive effect on long day plants and its failure on short day plants, Brain and his colleagues (1958-59) came out with a working model to explain the action of photoperiods on flower inducing substances. According to this model, during photo inductive red light treatment a precursor gets converted to
Gibberellin or Gibberellin like substance. The same substance is believed to undergo decaying back to the precursor either in dark or under far-red light treatment. According to their concept it is assumed that Gibberellin like substances have to be maintained at high concentrations in long day plants to be effective in producing the elusive compound called 'florigin'. But in short day plants, according to Brain, et.al. GA like substances are effective only when their concentration is very low for higher concentration of GA is inhibitory. That is why when GA is applied to short day plant there is no effect in terms of flower induction.

However, the said scheme of events fails to explain how low levels of GA like substances can produce sufficient quantities of florigin in short day plant to bring about the induction of flowers and it is expected that the florigin that is produced in short plants or long day plants should be the same.

Even today, with all the knowledge of molecular biology of the exact processes involved in inducing flowers are not known. Still, it is very important to understand the model proposed by Chailkhyan, a great Russian plant scientist. He toiled his entire life time to understand this phenomenon and his proposed model is very worth understanding. Cajlachjan, another way to pronounce his name, has assumed that the florigin formation takes place at two levels but in two steps. Further, florigin is not one substance but it is a complex of two substances, i.e., gibberellins and Anthesins. It is also assumed that long day plants synthesize anthesins irrespective of photoperiods, which means anthesins are produced constitutively, which indicates that the genes responsible for the synthesis of the above said compound are constitutively expressed all the time in long day plants. But the synthesis of GA or GA like compounds is under the control of long day photoperiodic conditions. In the sense, the pFR produced in long day plants has an important role in activating the pathway of GA synthetic. On the other hand, short day plants are believed to synthesize GA constitutively and the synthesis of anthesins is under the control of short day photoperiods. It means the pR form of the pigments is effective in inducing the synthesis of anthesin.

Gibberellin pathway and Light quality pathway. Each of them has certain products and they get integrated at the base of the meristem and determine and differentiate the floral meristem. Many of the components that are synthesized in floral meristem or axis, very young leaf primordials ultimately interact and integrate in activating the apical Meristem into floral meristem.

14.3. ábra - Figure 74. The role of GA in flowering
Light has another discerning, but subtle activity is inducing the synthesis of GA1 and its derivatives that activates LFY, and hypocotyl elongation and shade avoidance processes.

6. 14.6 Temperature can alter the flowering response to photoperiod

There are many examples of interactions between temperature and photoperiod, particularly with respect to flowering behavior (see Salisbury, 1963, for an extensive listing). In most cases, the interaction results in relatively subtle changes in the length of the critical photoperiod or a tendency toward daylength neutrality or an inability to flower altogether at high or low temperature extremes. There are other plants, however, for which flowering is either quantitatively or qualitatively dependent on exposure to low temperature. This phenomenon is known as vernalization. Vernalization is a means of preventing precocious reproductive development late in the growing season, ensuring instead that seed production does not begin until the beginning of the next growing season so that the seed will have sufficient time to reach maturity. Vernalization refers specifically to the promotion of flowering by a period of low-temperature and should not be confused with other miscellaneous effects of low-temperature on plant development. The term itself is a translation of the Russian yarovizatsya; both words combining the root for spring (Russian, yarov; Latin, ver) with a suffix meaning “to make” or “become.”

Coined by the Russian T. D. Lysenko in the 1920s, vernalization reflects the ability of a cold treatment to make a winter cereal mimic the behavior of a spring cereal with respect to its flowering behavior. The response had actually been observed many years earlier by agriculturalists, but didn’t receive critical attention of the scientific community until J. G. Gassner showed in 1918 that the cold requirement of winter cereals could be satisfied during seed germination. For his part, Lysenko received considerable notoriety for his conviction that the effect was an inheritable conversion of the winter strain to a spring strain. His position—a form of the thoroughly discredited Lamarkian doctrine of inheritance of acquired characteristics—was adopted as Soviet dogma in biology and remained so until the 1950s. The adoption of Lysenko’s views as official dogma had a significant impact on Soviet biology and placed agriculture in the USSR at a severe disadvantage for decades.
7. 14.7 Vernalization occurs most commonly in winter annuals and biennials

Typical winter annuals are the so-called “winter” cereals (wheat, barley, rye). “Spring” cereals are normally daylength insensitive. They are planted in the spring and come to flower and produce grain before the end of the growing season. Winter strains, however, if planted in the spring would normally fail to flower or produce mature grain within the span of a normal growing season. Winter cereals are instead planted in the fall.

They germinate and over-winter as small seedlings, resume growth in the spring, and are harvested usually about midsummer. The over-wintering cold treatment, or vernalization, renders the plants sensitive to long days (Figure 75.).

14.4. ábra - Figure 75. The effect of vernalization on plant development

One of the most thorough studies of vernalization and photoperiodism was carried out on the Petkus cultivar of rye (Secale cereale) by F. G. Gregory and O. N. Purvis, beginning in the 1930s. There are two strains of Petkus rye: a spring strain and a winter strain. The spring strain of Petkus rye is a facultative long-day plant. Under short days, floral initiation does not occur until after about 22 leaves have been produced, typically requiring a season of about 4.5 months. Under the appropriate long-day regime, however, flowering in the spring strain is initiated after as few as seven leaves have been produced, requiring only about two months.

When sown in the spring, the winter strain is insensitive to photoperiod. The winter strain flowers equally slowly - requiring four to five months - regardless of daylength.

If seeds of the winter strain are sown in the fall, however, the germinated seedlings are subjected to an over wintering low-temperature treatment. When they resume growth in the spring, winter strain plants respond as long-day plants in the same way as the spring strain. The effect of the over wintering cold treatment can also be achieved by vernalizing the seed, that is, by holding the germinated seed near 1°C for several weeks. Note that the low-temperature treatment, at least in the case of winter annuals, does not alone promote early flower initiation. Rather, the effect of vernalization is to render the seedling sensitive to photoperiod. Another example of vernalization is seen in biennial plants. Biennials are monocarpic plants that normally flower (and die) in the second season, again following an over-wintering cold treatment. Typical biennials include many varieties of sugar- and table-beet (Beta vulgaris), cabbages and related plants (Brassica sp.), carrots (Daucus carota) and other members of the family Umbellifereae, foxglove (Digitalis purpurea), and some strains of black henbane (Hyoscyamus niger). Biennials share with the winter annuals the property that subjecting the growing plant to a cold treatment stimulates a subsequent photoperiodic flowering response. Biennials typically grow as a rosette,
characterized by shortened internodes, in the first season. Over winter, the leaves die back but the crown, including the apical meristem, remains protected. New growth the following spring is characterized by extensive stem elongation, called bolting, followed by flowering. The cold requirement in biennials is qualitative (i.e., absolute). In the absence of a cold treatment many biennials can be maintained in the nonflowering rosette habit indefinitely. As a rule, vernalized plants, whether winter annuals or biennials, tend to respond as long-day flowering plants, although some biennials are daylength-indifferent following vernalization. One exception to the rule is the perennial Chrysanthemum morifolium, a SDP. Some varieties of Chrysanthemum require vernalization before responding as a quantitative SDP. As a perennial, Chrysanthemum normally requires vernalization on an annual basis. Many other plants such as pea (Pisum sativum) and spinach (Spinacea oleracea) can be induced to flower earlier with a cold treatment but it is not an absolute requirement.

8. 14.8 Literature

9. 14.9 Questions
1. Define phototropism. How do plants classify according to their photoperiodism-based flowering?
2. Describe the effect of GA on flowering!
3. What does the phase of vernalization mean?
4. What pigment(s) function as the photoreceptor for phototropism?
15. fejezet - 15. Formation of seeds and fruits

1. 15.1 Fruit set and development is regulated by hormones

The fruit is the final stage in the growth of the reproductive organ. Botanically a fruit is a mature or ripened ovary wall and its contents, although in some plants other floral parts may become involved. There is a wide diversity of fruits, depending on how the ovary develops.

In its simplest form, such as peas or beans, the fruit consists of the seed or seeds enclosed within an enlarged but dry ovary (the pod). Such fruits are classified as dehiscent fruits - dehiscent because at maturity the ovary wall breaks open to free the seeds. The fruit of Arabidopsis is a dry dehiscent fruit. Maize (Zea mays) is a non-dehiscent dry fruit consisting of a single seed with its seed coats fused with the dry ovary wall (a structure called the pericarp).

Tomato is an example of a fleshy fruit (actually a berry) with an enlarged, fleshy inner fruit wall. In some species, a structure other than the ovary wall develops as the fruit. These are called pseudocarpic fruits. A strawberry is one example. A strawberry “fruit” actually consists of a number of individual one-seeded fruits (called achenes) borne on the surface of an enlarged, fleshy receptacle. In many cases, it is clear that the fruit undergoes considerable cell division and cell enlargement as well as significant qualitative changes. These changes are due largely to changes in hormone content. Fruit development, maturation, and ripening have been widely studied because of their biological significance - fruits protect the developing seed and serve as a vehicle for dispersal of the mature seed – as well as their practical importance as a significant component in human nutrition. The development, maturation, and ripening of fleshy fruits have received the bulk of the attention over the years because of problems associated with transportation, storage, and other aspects of post-harvest physiology.

2. 15.2 The development of fleshy fruits can be divided into five phases

Tomato (Lycopersicon esculentum) has become a popular model in which to study fleshy fruit development, in part because there are numerous mutants available and the plant is easily transformed. With tomato as a model, the life history of a fruit can be divided into five more-or-less distinct phases. Phase I involves the development of the ovary in preparation for fertilization and seed development and ends with the decision to either abort further development or to proceed with further cell division and cell enlargement in the ovary walls. This decision to proceed with ovary development is generally referred to as fruit set. In phase II, or the initial phase of fruit development, growth of the nascent fruit is due primarily to cell division. The cells thus become small and dense, with very small vacuoles. During phase III, cell division effectively ceases and further growth of the fruit is mostly by cell enlargement. Once the fruit has reached its final size, it enters phase IV, or a period of ripening. In a fleshy fruit like tomato, ripening involves the development of color and flavor constituents (e.g., carotenoids, sugars, and acids) and a softening of the tissue that render the fruit attractive to animals. Tissue softening is due primarily to increased activity of enzymes such as polygalacturonase (PG). PG degrades the pectic substances that are found in the middle lamella and which are responsible for cell-to-cell adhesion. Finally, in phase V, senescence sets in and the fruit begins the decay process. As might be expected, all of the plant hormones are active at various stages during fruit development. During seed development and first and second phases of fruit development, auxins, cytokinins, and gibberellins are all present and active. Cytokinin level peaks during phase II, the period of most active cell division. Auxin level peaks in early phase III, coinciding with the initiation of cell enlargement, and then declines as the fruit reaches mature size. A second surge in auxin level occurs in the early stages of ripening, along with the appearance of significant levels of ethylene. The role of gibberellins is not well understood, but they are probably involved with cytokinins in initiating cell division and with auxin in maintaining cell enlargement. Tomato is a climacteric fruit and the burst in respiration is related to the appearance of ethylene and the qualitative changes in the fruit that represent ripening.
3. 15.3 Ripening is triggered by ethylene in climacteric fruits

In many, but not all, fleshy fruits the metabolic and visual changes that occur during the ripening process are accompanied by a significant burst in respiratory activity or CO2 evolution, called the climacteric. Examples of climacteric fruits include tomato, cucurbits (cucumber and related fruits), banana, apple, peaches, and plums. Nonclimacteric fleshy fruits, which do not show the CO2 burst, include strawberry, grape, citrus, and all nonfleshy, or dry fruits such as Arabidopsis or maize. The ripening process in climacteric fruits has attracted a lot of research over the years because of its economic importance and because just prior to the respiratory burst there is a significant increase in the production of ethylene. Moreover, ethylene synthesis is also auto-catalytic. Once ethylene production begins in one fruit, its production is stimulated in surrounding fruit—hence the old axiom that one rotten apple spoils the barrel. The role of ethylene in fruit ripening has assumed significant commercial importance. For example, tomatoes, bananas, and other climacteric fruits that have to be shipped any distance are commonly picked at the mature green stage and then ripened at their destination by gassing with ethylene. The rate limiting steps in the biosynthesis of ethylene are catalyzed by the enzymes ACC synthase (ACS) and ACC oxidase (ACO). In tomato there are two ACS genes that are expressed in the fruit and appear to be responsible for triggering ripening. Both genes, LeACS1A and LeACS4, are under developmental control and are induced at the onset of ripening. Furthermore, the induction of both genes is impaired by mutations at either the ripening inhibitor (rin) or the nonripening (nor) locus. In other words, fruits of tomato plants carrying the rin and nor mutations do not produce ethylene, do not exhibit a climacteric CO2 burst, and do not ripen. The expression of the gene LeACS4 is also controlled by ethylene itself and thus appears to be responsible for regulating the autocatalytic production of ethylene by a positive feedback system. A large number of ethylene signaling components have been identified in both Arabidopsis and tomato, including some ethylene receptors that are present only in the fruit and are strongly induced during the ripening process. The challenge now is to understand how these many components interact to form a coherent signal transduction chain that regulates a multitude of fruit-ripening genes.

4. 15.4 Literature


5. 15.5 Question
1. Which hormone regulates the fruit development?