

Molecular plant physiology

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MOLECULAR PLANT PHYSIOLOGY

lecture notes

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Preface

Plants are for the benefit of mankind since long as they are widely used as food and feed, raw materials for industry, fossil- or renewable source of energy. They produce oxygen and fix CO₂, clean air and contaminated soil and can be used as source of medically active substances. Although several basic genetic, biochemical and physiological processes are common in all the living organisms, plants have lots of specificities. They only require light, H₂O, CO₂ and mineral nutrients to live, but due to a sessile lifestyle they are forced to continuously adapt to their ever-changing environment.

The *Plant Molecular Physiology* textbook is designed to introduce undergraduate students into the life of plants. First, the genetic basis of the growth and development of plants is described. The first chapter explores the molecular regulatory factors with high importance in functioning of the genome and controlling the expression of genes. The second chapter introduces the specific organisation of plant cells and cellular organelles. Following chapters discuss the demand of plants for minerals, the uptake and transport of water and nutrients, and the mechanisms and significance of photosynthesis. The main endogenous factors (plant hormones) and the most important exogenous signal (light) and related signalling events affecting plant life and adaptation are also discussed. The plant-specific aspects of these biological processes, their regulatory and signalling mechanisms are also described. Then, the key points of plant ontogenesis including growth and development, reproduction and senescence are highlighted.

Understanding how plants function internally and how they adapt to their environment provide deeper insight into their unique and vulnerable life strategy that is essential to maintain the human civilization and the whole biosphere on this planet.

Expected learning outputs

Students are expected to gain the following knowledge, abilities, attitudes, autonomies and responsibilities learning these lecture notes and completing the related course:

Knowledge	Ability	Attitude	Autonomy/Responsibility
They know the characteristics of plants at molecular, cellular, physiological, and developmental levels.	They are able to use the obtained knowledge, the basic concepts and modern terminology of plant physiology correctly.	They are open to learn more about plants.	In front of professional or not-professional audiences, they autonomously argue for the essential role of plants in the functioning and maintenance of the Earth's biosphere.
They know the relationships between the plant's stationary lifestyle, development strategy and physiological functioning.	They are able to analyse differences between plant and animal development and adaptation strategies.	They do not consider plants as inferior to animals.	They draw others' attention to plants highlighting the uniqueness and appropriateness of their life strategies.
They know the most important plant growth regulators and their role in plant development and adaptation to the environment.	They are able to use the gained knowledge in scientific research and to generate new scientific results.	They are interested in a deeper understanding of plant life processes.	They independently discuss issues of plant life, development and environmental adaptation in front of a professional audience.
They know the importance of plant sciences in solving the burning problems of the nature and the human civilization.	They are able to use the gained knowledge to solve problems in plant protection, plant breeding, and plant cultivation.	They are open to learn about applied plant sciences.	They autonomously draw the attention of others to the importance of plants in everyday human life. They independently argue for the importance of plant sciences in solving current problems of the human civilisation and the biosphere.

Chapter 1. The organization and expression of the plant genome

This chapter introduces general and specific features of genetic information of plants that determines their phenotype, growth and functions. At first, basic terms used in molecular plant biology will be defined, followed by the description of the main structural elements of the genome and genes. A more detailed section deals with factors and regulatory mechanisms controlling gene expression. This store of learning supports understanding molecular and signalling mechanisms, processes of the growth and other physiological phenomena.

Learning goals:

- > Knowledge:
- The students know the specific features of the plant genome
- The students know the functional difference between the heterochromatin and euchromatin and mechanisms responsible for their transition
- The students know the levels of gene expression regulation
- > Abilities:
- The students can rightly use plant molecular biology terms
- They are able to use the obtained knowledge to understand the role of gene regulatory processes in plant development and adaptation
- > Attitude:
- The students are open to carry out molecular biology experiments with plants
- The students recognise the uniqueness of plants at the molecular level
- Autonomy/responsibility
- The students can autonomously study molecular processes in plants
- The students can independently argue why and how plants are similar or different at the genome organisation/gene regulation level as compared to animals

Plants, like all living organisms except certain viruses, contain the information required for their growth and functioning in the form of DNA. The total DNA content of a cell is known as its genome. The genome means the total DNA content of chromosomes in a haploid cell (1n). Diploid cells contain two sets of homologue chromosomes, two genomes.

Gregor Johann Mendel in the 1850s conducted a series of experiments with pea (*Pisum sativum*) and concluded that some physical traits (phenotype) are determined by heritable components. These heritable factors, responsible for e.g. the plant height, shape and colour of seeds and pods, flower position and petal colour, are called genes. Gene is the DNA region that contribute to the mature mRNA used to produce a protein (or RNA) and regulatory regions surrounded by other non-coding sequences and regulatory sections. According to the present term the regulatory sequences with important roles in the timing and extent of the

transcription are part of the gene, even if their distance from the coding region may be frequently several hundreds or even thousand pairs of nucleotide bases (bp) [1].

1.1. Organization of plant genomes

The plant genome consists of the nuclear genome, which contains majority of the genes necessary for plant development and growth, and the genome of organelles (1-10%). Mitochondria and plastids have a subset of genes required for their own functions; they have the capacity to synthesis proteins under a strict control of nuclear genome [2]. The nuclear genome of plants is organized into chromosomes, their numbers in higher plants are between 10 and 48. During the evolution occurred several times genome duplication. Multiplication of the chromosomes of the same species resulted in autoployploid plants, while plants bearing chromosomes from closely related species are called allopolyploids (for example Brassicaceae species are allotetraploid, wheat is allohexaploid). Following the ancestral genome duplications, the plants usually became diploid (the DNA contents of the macro- and microspores became double, $2n \rightarrow 1n$). Genes present in multiple copies might be loss, change their function or expression pattern [1]. However, in plant somatic cells frequently occur endoreduplication, thus sometimes 4n, 8n, 16n and even bigger genome number can be found in the older, larger or specified cells of one organism [3].

The plant genome may cover a wide range of size. While *Paris japonica* has the largest known eukaryotic genome $(1.5 \times 10^{11} \text{ bp})$, the *Genlisea tuberosa* (a carnivorous species endemic to Brazil) [4], has the smallest known plant genome $(6.1 \times 10^7 \text{ bp})$. The human genome lies in the middle of this range with the 3×10^9 bp. These are good examples for the so-called C-value paradox, which says that the genome size of an organism does not correlate directly with its complexity (C-value is the DNA content of the haploid cell). Interestingly, the number of genes encoding proteins does not differ so much among plants: their estimated number is between 30 000 and 60 000 [2].

Generally, only 1-10% of the whole genome is transcribed to mRNA and translated to protein. In the coding sequences can be classified single-copy DNA, large multigene families where genes occur in 20- 50 repeats, or gene cluster and tandem repeats that typically code for gene products in great demand. Furthermore, there are non-protein-coding RNA-coding genes, which are heterologous sequences with usually regulatory role. The first sequenced genome among the higher plants was that of *Arabidopsis thaliana*, the model plant of molecular plant research [5]. *Arabidopsis* has 5 pair of chromosomes, 1.19x10⁸ bp nuclear genome (totally 1.35x10⁸ bp). According to the latest data (www.arabidopsis.org) [6], it contains 27655 protein-coding genes, more than 5000 non-protein-coding (they code usually rRNAs, tRNAs or other RNAs with regulatory function) and 4800 non-functional protein coding genes (e.g. pseudogenes).

Most of the genome sequences are non-coding or with unknown function or highly repetitive [7] (Biscotti et al. 2015). Repetitive DNA makes up much of the genome in many plants and they can be responsible for the big differences in the genome size even among the closely related plant species. Several different types of non-coding or repetitive sequences exist, such

as repeats in chromosome telomeres and centromeres and other, so-called satellite DNA, which can be classified further according to the size and position of the repeated sequences (e.g. simple sequence repeats, tandem repeats, dispersed repeats). Among the dispersed repeats are pseudogenes and non-functional gene sequences (with deletion, insertion or without promoters, etc.) which can be present in large quantities (their number in *Arabidopsis thaliana* is ca. 5000). Among the dispersed repeats can be found mobile genetic elements (MG elements) too.

MG elements are the transposable elements (TE), which are DNA-sequence elements that move or transpose from one site of the genome to another. According to the mechanism by which they transpose they are classified as transposons (supposedly originated from mRNAs) and retrotransposon (during the transposition process an RNA intermediate is synthesized; their origin is presumably viral). TEs were discovered by B. McClintock [8] in *Zea mays* (Ac/Ds system). Transposons constitute approximately 85% of the maize genome but their number can be also extremely high in some other species [9]. As an example, in rice the Tos 1-20 elements can be found in near 1000 copies. In some plants the TE elements make up the most abundant class of dispersed repetitive sequences. Most of them are inactive or their activity is lowered by different mechanisms in plants, because they may modify the expression of nearby genes [1]. In Arabidopsis 3900 TE genes were identified [6], which can be regarded to relatively low amount among the higher plants.

1.2.2. Structure of the chromatin

The long DNA molecule in the nucleus is packed with proteins to form chromatin. The proteins are important both in maintaining the structure of a chromosome and in regulating gene expression. Investigating the chromatin using high resolution microscopy its structure resembles 'a beads on a string' [2]. 'Beads' are the nucleosomes, which contain 166 bp of DNA wrapped around a globular octamer (two tetramers of H2A, H2B, H3 and H4) basic histone proteins (Fig.1.1.). H1 resides outside of the core, it links coils consisting of 6 nucleosomes thus stabilizing the higher-order chromatin structures. Based on cytological observations of how darkly the chromatin is stained, two forms can be distinguished: the regions of heterochromatin and euchromatin. The heterochromatin stains darkly with dyes, is tightly coiled and can be found often near the centromeres of the chromosomes. Generally, it is transcriptionally silent [1]. In contrary, the euchromatin is packed more loosely and characterized by less interacted H1 histones and by the presence of other specific proteins such as High Mobility Group (HMG) proteins. Binding of the HMG proteins may result in the blending of the double-stranded DNA molecule thus facilitates the attachment of other DNA-binding proteins.

Modification of histones in chromatin affects DNA accessibility for transcription. Especially H3 and H4 histones may undergo post-translational modifications which can affect gene expression both by altering chromatin structure and binding capacity of transcription factors and RNA polymerases that form the basal transcriptional machinery. (The transcription factors are proteins that may promote the forming of the transcriptional initiation complex and thus facilitate the gene expression.) The two major forms of modifications are acetylation and methylation. During acetylation, an acetyl group is transferred to one or more Lys amino acid

residues of H3 or H4 histones and in this form the nucleosome needs more space (Fig. 1.1.). The activity of histone acetyl transferases (HATs) eventuates a less compact, while histone deacetylases result in a more condensed chromatin structure, which is less accessible for transcription factors [1]. The histone demethylases also cause looser DNA structure and promote active gene transcriptions. In contrary with this, methylation of histone proteins by histone methyl transferases (HMTases) and DNA cytosines by DNA methyl transferases result in more condensed structure where the gene transcription is inhibited.



Fig. 1.1. Modification of histone proteins by histone acetyl transferases cause less condensed chromatin (A). In the looser chromatin region transcription factors can bind to the regulatory sequences of DNA (B) (modified from Erdei, 2011, with permission).

1.2. The plant genes

A typical plant gene contains several different regulatory sequences beside the region which is transcribed to RNA and encodes the gene's protein product (Fig. 1.2.). The DNA sequences surrounding the coding region contains structural and regulatory elements that have role in gene transcription and protein synthesis (translation). On the 5' region, some distance from the transcription start site (upstream) can be found the promoter. The core or minimum promoter region involves the most important sequences that are crucial in the transcription, like TATA-box, that has a function in the binding of the basal transcription factors (TFs) of the initiation complex and activating the RNA polymerase, while CAAT-box and GC-box are the binding sites of other TFs. The 5' region of the gene (upstream) contains further regulatory sequences (*cis*-regulatory elements) which can modify the process of transcription. Moreover, regulatory information is present also in the coding region. Intervening sections of protein-coding regions are introns, while the sections encoding protein product are exons (Fig. 1.2.). Most of the plant genes contain both exons and introns. The number of introns in a typical plant gene is usually low, but it can reach even 40 and their total length may exceed the amino acid-coding exons' total length [2].



Fig. 1.2. The structure of a typical protein-coding plant gene and the main processes determining the protein synthesis. The DNA sequences of the gene contain both 5' and 3'flanking regulatory sequences, such as *cis*-regulatory elements of the promoter or polyadenylation signal. The primary transcript (mRNA precursor) is larger than the protein-coding regions. The non-protein-coding intron sequences will be removed during the mRNA maturation process (modified from Erdei, 2011, with permission).

1.3. Regulation of the gene expression

1.3.1. General features

The mechanism and regulation of plant's gene expression basically do not differ from that of other eukaryotes: transcription takes place in the nucleus, while the translation in the cytoplasm. The transcription is catalysed by RNA polymerases (RNAPs) build-up from 8-12 subunits. Plants have five different nuclear RNAPs, each of them is involved in synthesis of different types of transcripts. The RNAP I is responsible for the transcription of 28S rRNA, 18S rRNA and 5,8S rRNA; the RNAP II for the mRNA precursors of protein-coding genes, but beside those even some other RNA-coding genes, such as microRNA- and small nuclear RNA genes are transcribed by it; the RNAP III catalyses the 5S rRNA and tRNA synthesis. Plants contain another two, plant-specific RNAPs, called RNAP IV and RNAP V, which in their two biggest subunits (RNPD1, RNPD2) differ fundamentally from RNAP II. RNAP IV is involved in endogenous small interfering RNA (siRNA) synthesis and RNAP V in the transcription of repetitive sequences [1]. Binding of RNAPs to DNA requires the presence of several general TFs (e.g. for RNAP II the TFIIA, B, D, E, F, H and the TATA-binding protein vital in recognition of TATA-box are important; see Fig. 1.3.).

Plants, as other organisms require several protein products only in specific cells and tissues or in particular developmental stages. The expression of many plant genes changes during

development or in response to environmental factors [1]. The timing and extent of gene expression are controlled by the promoters and other regulatory elements. For example, there are promoters that determine the specific expression of genes in mesophyll cells or others in guard cells of leaves, and similarly, specific promoters are known responsible for root- or tuber-specific gene expression patterns.

1.3.2. Cis-acting regulatory elements

Regulatory elements found in the DNA strand called *cis*-acting elements. *Cis*-elements are usually short sequence regions (<10 bp) and vary greatly in their base composition [1]. They can be either enhancers that increase the efficiency of RNAP II in initiation of transcription (frequently located at a considerable distance from the coding region), or silencers that downregulate the transcription. Several specific *cis*-acting regulatory elements of promoters were identified defining the specificity of gene expression. The *cis*-elements responsible for gene expression regulation of hormones and other signalling molecules are the 'response elements'. There are enhancer elements that increase the transcription of genes and silencers which lower the transcriptional activity. The *cis*-elements can occur in several copy in the promoter region of one gene amplifying their effect, but they can act even individually. In regulatory region of genes encoding proteins with essential function and high demand (like actin, tubulin genes) can be found *cis*-elements ensuring high level of expression [1].

1.3.3. Transcription factors

The transcriptional initiation complex can interact with several proteins. The *cis*-elements of promoters and other regulatory regions can modify the gene expression via transcription factors. The TF proteins usually synthesized independently on genes of which transcription they influence and are trans-acting elements. TF proteins bind to the regulatory sequences found in the DNA. Depending on their domains, TFs can be either transcriptional activators or repressors. While the activators increase the expression of the gene(s), repressors block the effects of activators, decrease the rate of transcription, generally via binding to silencers (Fig. 1.3.). The activators and repressors can switch 'on' or 'off' a gene. Their effect on transcriptional machinery is mediated by other proteins called co-activators or co-repressors [10]. Their combination is different at each gene and they may be subjects of different types of regulation, too. The Plant Transcription Factor Database (http://planttfdb.gao-lab.org) contains 58 TF families [11]. The TFs can be grouped also by their structural motifs (such as helix-turn-helix, helix-loop-helix, basic leucine zipper, zinc finger). Several TFs are specific for plants. As an example for their importance, TFs containing homeobox domains regulate development and determine cell fates. Genes, that regulate tissue- or organ identity are called homeotic genes. Their encoded proteins control the expression of other regulatory proteins, including other TFs, and thereby act as 'master genes' [2].



Fig. 1.3. The transcription apparat in eukaryote cells. Beside the RNA polymerase II (RNAP II), several general transcription factors are essential (TBP, TATA-binding protein, TFIIA-H). Among the proteins of the transcription complex can be found activators and repressors which bind to specific regulatory DNA sequences, which are the enhancer and silencer elements, respectively (modified from Erdei, 2011, with permission).

1.3.4. The heterochromatic small interfering RNAs

Recently, small regulatory RNAs (sRNAs; approximately 20-24 nt in size) have emerged as important gene expression regulators [12]. RNA-mediated transcriptional gene silencing (TGS) is a conserved phenomenon that occurs in fungi, plants and animals [13]. In plants, the predominantly 24-nt long heterochromatic small interfering RNAs (het-siRNAs) transcriptionally regulate gene expression by RNA-directed DNA methylation (RdDM). RdDM silences transposable elements and repeat sequences and in this way they have crucial roles in maintaining the genome stability. The number of plant het-siRNAs can be extremely high (more than 10 000). They usually derived from transposable elements and repeats. The plant-specific RNA polymerase IV (RNAP IV) produce single-stranded RNAs from the target sequences, then they are converted to double-stranded RNAs by an RNA-dependent RNA polymerase (RDR2). The double-stranded RNAs are processed by a complex to 24-nt siRNA duplexes. The mature siRNAs then associate with sRNA-binding ARGONAUTE protein (AGO4, -6 or -9) and interacts with scaffold RNAs transcribed from the target loci by the RNA

polymerase V (NRAP V). This interaction guides DNA methylation (histone H3K9 methylation) to result in the silencing of the target sequences. As a consequence, heterochromatin formation may occur [13]. The DNA methylation also may result in epigenetic changes, because specific mechanisms ensure the inheritance of DNA methylation patterns both mitotically and meiotically. The epigenetic mechanisms control many cellular and developmental processes during plant development [1].

1.4. Other mechanisms controlling gene expression

1.4.1 Post-transcriptional regulations

During the transcription the whole exon-intron region of DNA is transcribed to RNA, that is the mRNA-precursor (Fig. 1.2.). The primary transcript undergoes different modifications such as splicing (when the introns will be removed), addition of the 5' "cap" structure and 3'polyadenylic acid end (poly A tail). These modifications promote that the mature mRNA can reach the ribosome and may translated to the entire protein [2]. However, there are known some other posttranscriptional regulations and among them several include sRNAs. The two most important sRNA classes in this process can be the microRNAs (miRNAs) and the plant-specific phased siRNAs (phasiRNAs); the latter group belongs to the secondary siRNAs [14, 15].

The microRNAs are transcribed by RNAP II from the *MIRNA* genes and their biogenesis is a widely conserved process in plants. Plant genome typically encodes a hundred to several hundreds of *MIRNA* genes which can be located intergenic (between two protein-coding genes) or intronic [15]. The primary transcript (pri-miRNA) contains a self-complementary foldback (hairpin) structure. After slicing by an RNase III endoribonuclease (DICER-LIKE1), the miRNA/miRNA* duplex is methylated (which is crucial for their stability) and transported to the cytoplasm, where the mature miRNA incorporates into the ARGONAUTE protein (AGO1) forming the RNA-INDUCED SILENCING COMPLEX (RISC). The miRNAs, requiring almost perfect sequence complementary with its target, can trigger the cleavage of the mRNA of target gene (most usual mechanism for plant miRNAs) or inhibit their translation [16, resulting in gene silencing. This mechanism is known as RNA interference (RNAi). The target sequences of miRNA frequently are TFs, and in this way they have a wide range of roles both during normal development and stress responses of plants [2].

Phased secondary siRNAs belong to another class of sRNAs. Their biogenesis relies on the cleavage mediated by sRNAs (mostly miRNAs). There are two typical modes of their biogenesis which eventuate several 21-, 22- or 24-nt siRNAs through sequential miRNA-directed cleavage of one mRNA molecule. These phasiRNAs can function like miRNAs to regulate their target genes in *trans* (*trans*- acting siRNAs, tasi-RNAs) or in *cis* (casiRNAs). The known targets of tasi-RNAs are auxin response factors (ARFs) and having role in the auxin signalling elucidate their involvement also during the entire life of plants [16].

1.4.2. Post-translational regulations

The gene products can be regulated even post-translationally due to different protein modifications, which may affect their function and lifetime. During or after the completion of translation, the secondary and tertiary structure will be formed due to protein folding [1]. The

synthesised protein can be transported to membranes or specific organelles and processed e.g. by proteolytic cleavage. The molecule can bind co-factors, intra- and intermolecular disulfide bounds can be formed and additional functional groups or molecules can be attached, such as phosphoryl-, carboxyl-, acetyl groups, glucosides or ubiquitin.

Protein ubiquitination can result in altered function, localization or degradation via the 26S proteasome system. The protein degradation by the 26S proteasome is essential in plant hormonal signalling pathways. Conjugation of an ubiquitin to a substrate is carried out by a multienzyme complex and requires ATP. The enzymes generally involved in the process are called E1 (ubiquitin activating enzyme), E2 (ubiquitin conjugating enzyme) and E3 (ubiquitin ligase) (Figure 1.4). The protein in E3 complex which recognizes the substrate contains F-box motif. The number of F-box proteins in plants are very high because each of them interacts with a different protein or protein family substrates [1]. They regulate diverse cellular processes, including cell cycle transition, transcriptional regulation and signal transduction. In *Arabidopsis thaliana* genome the presence of at least 568 F-box protein genes were reported [17].



Fig. 1.4. The mechanism of ubiquitin-ligase complex mediated protein degradation. (Figure of A. Fehér)

Summary

- 1. The main characteristic parameters of a species are determined by its DNA. The genome is the total DNA content of a haploid cell. Plants have nuclear, mitochondrial and chloroplastic genomes. Most of the higher plants are diploids, although the polyploidy and genome duplication are frequent phenomena.
- 2. The size of plant's genome varies greatly. Plants having bigger genome have more repetitive DNA. In some plants the transposable elements (TE) make up much of the nuclear genome. The activity of repetitive sequences is suppressed by different mechanisms.
- 3. The nuclear DNA and histone proteins form chromatin The heterochromatin is a more condensed, transcriptionally inactive, while the euchromatin has a loose structure where transcription factors can bind. Modifications of DNA and histone proteins affect the DNA accessibility for transcription. Histone acetylation and -demethylation result in a less compact, while histone de-acetylation and -methylation a more condensed chromatin structure.
- 4. Plant genes are organized like those of other eukaryotes: the protein-coding region is interspersed and surrounded by regulatory sequences. Regulatory elements found in the DNA strand are *cis*-elements. *Cis*-acting elements responsible for regulation of hormones and other signalling molecules are called "response elements" The enhancer elements increase transcription, the silencers lower it. *Cis*-elements modify the gene expression through transcription factor proteins which can be activators or repressors. TFs are *trans*-acting regulatory elements.
- 5. The heterochromatic small interfering RNAs are transcriptional regulators promoting heterochromatin form. They silence transposable elements and repeat sequences by RNA-directed DNA methylation. In their biogenesis and mechanisms, the plant-specific RNA polymerase IV and RNAP V and the ARGONAUTE proteins are involved. The DNA methylation may result in epigenetic changes too.
- 6. The microRNAs are post-transcriptional regulators. They are transcribed from the *MIRNA* genes by RNAP II. After their processing, they build in the RISC (RNA-induced silencing complex) and trigger the cleavage of the mRNA of target gene or inhibit its translation. The resulted gene silencing mechanism is called RNA interference (RNAi). Their target sequences are frequently TFs.
- 7. miRNAs also have role in the biogenesis of phased secondary siRNAs. The pha-siRNAs originated from a long dsRNA molecule due to sequential cleavage. The 21-, 22- or 24- nt siRNAs regulate their target genes by mRNA cleavage. One of their group is the plant-specific *trans*-acting siRNAs (tasi-RNAs). The only known targets of tasi-RNAs are auxin response factors (ARFs), thus they have role in the plant hormone auxin signalling.
- 8. Protein ubiquitination is post-translational regulatory mechanism. The protein degradation by the 26S proteasome is essential in plant hormonal signalling pathways.

Questions

- 1. What is the difference between polyploidy and alloploidy?
- 2. Why can be big differences in the genome size of plants that are relatives?
- 3. What kind of sequence types are in the genome?
- 4. Compare the *cis* and *trans*-acting regulatory elements! What are the main similarities and differences between them?
- 5. Which mechanisms regulate the gene expression through modification of chromatin?
- 6. How act the miRNAs?
- 7. What plant-specific regulatory mechanisms control the amount of a gene product?

Questions to discuss

- 1. What can be the advantages of the multiplicated genomes in the somatic cells of the plants? Why is rarer it in macro- and microspores?
- 2. The *Paris japonica* is a plant with very slow growing and can be found very rarely in the nature. Can it be related with its genome size?
- 3. If the size of the genome does not correlate with the complexity of the organism than what can be related with the advanced state?
- 4. What can be the reason that so many heterochromatic siRNAs are in plants?

Suggested reading

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Chapter 2. An introduction to plant cells

This chapter introduces the organelles of plant cells (see Fig. 1). It starts with a discussion of the distinctions among cell wall types. This is followed by a short introduction of plant organelles and their roles in growth and development. The chapter details the structures of the endomembrane system. The functions of plastid types are also briefly surveyed.

Learning goals:

- > Knowledge:
- The students know the plant cell organelles
- The students can distinguish among the organelles of the various plant cell types
- The students know the differences between animal and plant cells
- > Abilities:
- The students can recognise plant cell organelles in microscopic images
- The students are able to use the obtained knowledge to understand the role of cell types/organelles in plant development and adaptation
- > Attitude:
- The students are open to carry out cell biology experiments with plants
- The students recognise the uniqueness of plants at the cellular level
- Autonomy/responsibility
- The students can autonomously study cellular processes in plants
- The students can independently argue why and how plants are similar or different at the cellular level as compared to animals



Fig. 2.1. An idealized plant cell and its components. (Source: Wikimedia Commons. This work has been released into the public domain by its author, LadyofHats. This applies worldwide. Link: https://commons.wikimedia.org/wiki/File:Plant_cell_structure-en.svg)

2. 1. Plant cell wall

The main difference between plant and animal cells is the presence of the cell wall. Their rigid cell walls take part in the development, which depends on the cell wall-mediated cell division and cell enlargement. Cell wall structure is diverse containing a variety of compounds. There are two main types of plant cell wall, the primary and the secondary cell wall. The major component of the fundamental framework of primary cell walls is the cellulose. These linear cellulose molecules can bond to each other by hydrogen bonds in order to form microfibrils. A microfibril can contain about 36 cellulose molecules. These cellulose rods can have connected to each other by cross-linking glycans and all this network is embedded in a matrix of pectic polysaccharides. Structural proteins or phenylpropanoids can be found as a third, independent and non-polysaccharide network. Callose is a compound which can be found in cell walls of pollen grains, pollen tubes or in the cell plates of dividing cells.

2.1.1. The primary cell wall

Cellulose microfibrils in the primary cell wall are relatively short and thin, compared to those of the secondary cell wall and hemicellulose is composed of xyloglucan. This type of cell wall is rich of pectin. Type I cell wall is specific for eudicot species and half of the monocots.

On the contrary, the type II cell wall is the main type of commelinoid monocots (bromelias, palms, sedges, grasses). During cell expansion, the biosynthesis and assembly of primary cell walls can occur. pH and other cell-wall loosening factors can contribute to the cell enlargement processes.

2.1.2. Secondary cell wall

The secondary cell wall is deposited between the primary cell wall and the plasma membrane. This type of cell wall contains relatively long and thick cellulose microfibrils, hemicellulosic xylan, and lignin. Among lignin, other depositions also occur like phenylpropanoids, suberin, cutin, or waxes. The secondary cell wall modifications have a lot of different functions as in the case of cotton fiber, pear fruit stone cells, collenchyma cell in the cell corners or guard cell pairs.

Middle lamella is a thin layer of material, consisting mainly of pectins, that binds together the walls of adjacent plant cells.

Apoplast is the name of the cell walls of the plant cell. It has important function in sensing the outer environment.

2.2. Membranes of the Cell-Endomembrane System

Plant cell and cell organelles are bounded by lipid bilayer membranes. Protoplast is the living part of the cell surrounded by plasma membrane without cell wall, which can separate it from external environment. Plant cells contain around 14 different membrane types.

2.2.1. Plasmodesmata

Plasmodesmata are tubular extensions of the plasma membrane, 40 to 50 nm in diameter, that traverse the cell wall and connect the cytoplasms of adjacent cells. Most plant cells are

interconnected by this structure, so their cytoplasms form a continuum referred to as the symplast. Symplastic transport is the way with which solutes can transport between cells.

Primary and secondary plasmodesmata help to maintain tissue developmental gradient primary plasmodesmata can form between clonally derived cells by cytoplasmic connections. There is a size exclusion limit which can restrict the transported molecules by size. This size is adjusted by the width of the cytoplasmic sleeve that surrounds the ER tubule, or desmotubule, which is the centre of the plasmodesmata. Globular proteins within the sleeve generate spiralling microchannels through the plasmodesmata. There is little information about the transport of solutes through the cytoplasmic sleeve and the desmotubule itself. Actin and myosin are located in the plasmodesmata. Viruses can also go across the plasmodesmata by movement proteins. These proteins can form a transport tubule within the plasmodesmatal pore that facilitates the mature viruses through the plasmodesmata.

Symplastic transport can also occur between non-clonally related cells through the formation of secondary plasmodesmata. In these connections, the plasma membrane of the adjacent cells can fuse and the ER network became connected. These facts can describe the importance of symplast in developmental signalling and nutrition.

2.2.2. Vacuoles

Vacuole is a membrane-enclosed compartment which contains vacuolar sap composed of water and other solutes. Large vacuoles which can located in central position can occupy up to 95 % of the total cell volume, so it can take role in cell expansion. The number of vacuoles varies between cell types, as in the case of flower petals with vacuoles. Vacuoles can differ in size and appearance; some stress types can change their size. The level of maturation also a factor which can determine the size of the vacuoles, e.g. there is no large central vacuole in meristematic cells, but they have many small vacuoles.

Tonoplast is the name of vacuolar membrane, which contains proteins and lipids that are synthesized initially in the ER. Vacuole is a storage compartment of the cell full with plant secondary metabolites involved in plant defence against herbivores and pathogens.

There are two types of vacuoles in plant cells. Lytic vacuoles are large, water-containing vacuoles, playing a role in water and ion balance. Variety of specific membrane transporters can contribute to the accumulation of inorganic ions, sugars, organic acids, and pigments or toxins. In seeds, there are protein bodies, which can accumulate proteins. Protein storage vacuoles are smaller and filled with proteins or lipids and they can be found in seeds.

2.2.3. Endoplasmic reticulum (ER)

ER is a network of internal membranes and the place of protein and lipid synthesis. In ER, proteins can be synthesized and delivered to the plasma membrane, or apoplast, vacuoles. ER has a quality control system. By multiple processing steps it can supervise and conduct the secreted proteins. ER is one of the largest calcium stores participating in the intracellular calcium signalling. It can identify and transfer the misfolded proteins to the ERAD machinery (ER-associated degradation).

ER is composed of tubules which can form flattened saccules called cisternae. Tubular and cisternal forms of ER rapidly transform to each other. This transition is regulated by a class of proteins called reticulons. Reticulons (RTNs) are membrane-spanning proteins sharing a typical domain named reticulon homology domain (RHD). RTN genes have been identified in all eukaryotes examined so far, in plants as well. RTZNs are involved in numerous cellular processes such as apoptosis, cell division and intracellular trafficking.

The region of the ER that contain many membrane-bound ribosomes is called rough ER, as it has rough appearance in electron micrographs. The ER without bound ribosomes is called smooth ER. Secretion of the proteins from cells begins with the rough ER. ER provides the building stones of membranes and protein cargo for other compartments in the endosomal system. Many proteins are synthesized on the rough endoplasmic reticulum. ER is the major source of membrane phospholipids. Flippases are enzymes which can counteract the membrane asymmetry by flipping newly synthesized phospholipids across the bilayer. ER and plastids are capable to add new membrane directly through lipid and protein synthesis. Smooth ER participates in fatty acid modification, lipid synthesis and the production of oil bodies.

2.2.5. Golgi apparatus

The Golgi apparatus processes and packages newly synthesized macromolecules. Glycoproteins and polysaccharides destined for secretion are processes in the Golgi apparatus.

Golgi apparatus has another name dictyosome. This organelle is a group of polarized stack of cisternae, with fatter cisternae on the cis side or forming face, which connect to the ER. The opposite face is the trans side of Golgi body is more flattened, thinner cisternae and include a tubular network called the trans-Golgi-network or TGN. Meristematic cells can contain up to hundreds of Golgi bodes, while other cells differ in their number of the Golgi bodies. Cisternae can be different in one Golgi body with different enzymes. There are some glycoprotein modification enzymes. Membrane and its content can be delivered to the Golgi from the ER at specialized site called ER exit sites (ERES). This site is determined by the presence of a coat protein called COP II. This can associate with the transmembrane receptors which bind the specific cargo destined for the Golgi. These membrane regions then bud off forming coated vesicles losing their COP II coats. Anterograde (forward) movement is the pathway out of the ER to the Golgi, within the Golgi from the cis to trans face, followed by transport to the plasma membrane or to the prevacuolar structures via vesicles. Contrarily, retrograde or backward movement is the way of recycling membrane vesicles from the Golgi to the ER or from the trans to cis face of Golgi

The main function of Golgi apparatus is the processes and packages polysaccharides and proteins for secretion.

Nowadays, the distribution of the Golgi stacks in a variety of plant cell types can be examined by immunohistochemical studies and by live cell imaging after expression of Golgi targeted fluorescent protein constructs, so the structure can be examined.

2.2.6. Trans Golgi network (TGN)

TGN is an important hub site in plant cells. TGN is essential for the assembly of cell walls, including the cell plate, and organizes traffic or cargoes not only to but also from the plasma membrane. TGN is a membrane compartment on the trans-side of Golgi stacks responsible for the sorting and packaging of cargo molecules for delivery to the plasma membrane and vacuoles. TGN is a distinct organelle and not just a tubular reticulum on the trans-side of the Golgi.

There are two forms of TGN, 1, the Golgi-associated TGN (GA-TGN) cisternae attached to the trans-side of the Golgi; and 2, the detached, free TGN cisternae.

2.2.7. Microbodies

Microbodies are the site of specific biochemical pathways.

Some organelles grow and proliferate independently from the endomembrane system even though they can form from that. These organelles are oil bodies, peroxisomes and glyoxysomes.

During seed development, many plants store large amounts of oil, which can be accumulated in organelles called oil bodies, lipid bodies, or spherosomes. They are surrounded by a halfunit membrane, a phospholipid monolayer. Oil bodies are formed within the ER. They can store triglycerides, so their lumen is hydrophobic.

When oil bodies can degrade during seed germination, they can associate with other organelles that contain the enzymes for lipid peroxidation, so the glyoxysomes.

Microbodies are spherical organelles are surrounded by single membrane and they are specialized for one special functions.

Peroxisomes and glyoxysomes are microbodies that are specialized for the B-oxidation of fatty acids and the metabolism of glyoxylate, a two-carbon acid aldehyde. Microbodies lack DNA and they can associate with other organelles in order to share intermediate metabolites. The glyoxysome is associated with mitochondria and oil bodies, while the peroxisome is associated with mitochondria and oil bodies.

Peroxisomes can develop directly from glyoxysomes, at least in greening cotyledons.

In the peroxisomes, glyoxylate, a two-carbon oxidation product of the photorespiratory cycle is oxidized to the acid aldehyde glyoxylate. In this reaction, hydrogen peroxide can generate. The most abundant enzyme located in this organelle is the catalase, which can degrade the hydrogen peroxide. Catalase enzyme can exist in crystalline forms.

Most proteins can enter the peroxisomes from cytosol posttranslationally by means of a specific targeting signal, consisting of serine-lysine-leucine at the carboxyl terminus.

2.3. The nucleus

The nucleus is the organelle which contains the genetic information responsible for regulating the metabolism, growth, and differentiation of the cell. Nuclear genome refers to the genes and their intervening sequences. The size of the nuclear genome is very different between

plants. *Arabidopsis thaliana* has a genome size around 1.2x10⁸ base pairs, while the lily *Fritillaria assyriaca* has 1x10¹¹ base pairs. Chloroplasts and mitochondria have own genetic information.

Nucleus has double membrane called nuclear envelope, this is a subdomain of the endoplasmic reticulum (ER). There are some selective channels across both membranes which connect the nucleoplasm with cytoplasm called nuclear pores. The number of these types of pores can vary between hundreds to thousands. These nuclear pores can be composed of more hundred different proteins called nucleoporins in order to form a 105-nm long nuclear pore complex. The NPC can form a 40 nm long channel and act as a supramolecular sieve. The nuclear localization signal is a specific amino acid sequence which is required for a protein to enter into the nucleus.

Chromosomes can store and replicate in the nucleus. Chromosomes can compose of DNA and its associated proteins, this DNA-protein complex is called chromatin. Segments of the linear double helix of DNA are coiled twice around a solid cylinder of eight histone protein molecules, and this structure is called nucleosome. At interphase, two types of chromatin can be condensation: distinguished on different heterochromatin and euchromatin. Heterochromatin is a highly compact and transcriptionally inactive form of chromatin which can count of about 10 % of DNA. Most of the heterochromatin is concentrated along the periphery of the nuclear membrane and is associated with the regions of chromosomes containing few genes, such as telomeres and centromeres. The rest of the DNA consists of the euchromatin which is dispersed and transcriptionally active form. At any given time just the 10% of euchromatin is active transcriptionally. Dynamic structural changes with chromatin during the cell cycle. Transient local changes are required for transcription and heterochromatic regions can be converted to euchromatic regions and vice versa by addition and removal of functional groups on the histone proteins. Such enormous changes globally the whole genome can result to stable changes in gene expression. Stable changes in gene expression can occur without changes in the DNAS sequence called epigenetic regulation.

Nuclei contain a dense region called nucleolus, which is the site of ribosome synthesis. Typical cells have one nucleolus while others have more. The nucleolus includes some chromosomes where ribosomal RNA (rRNA) genes are clustered to form a structure called the nucleolar organizer region. The nucleolus assembles the proteins and rRNA of the ribosome into a large and small subunit, which unite in the cytoplasm to a complete ribosome. These ribosomes are protein synthesizing factories.

2.4. Mitochondria

Mitochondria synthesize ATP and small carbon skeletons.

Mitochondrium is an energy-producing, semiautonomous and independently dividing organelle. It is separated from the cytosol by a double membrane (an outer and inner membrane) and contain their own DNA and ribosomes. Mitochondria are the site of cellular respiration, a process which can produce energy from sugar metabolism for ATP synthesis. Mitochondria can always undergo fission and fusion. The inner membrane contains proton-pumping ATP synthase which uses a proton gradient to synthesize ATP for cells. The

generation of proton gradient is the work of the electron transport chain, a massive assembly of electron transporters, which are located in the inner membrane.

Cristae are the enfolded inner membranes in plant cells. The inner membrane can mean a border for the mitochondrial matrix, the place where the enzymes of Krebs cycle are located.

2.5. Plastids

Plastids are a diverse family of anabolic organelles. Plastids cannot be formed de novo in the cytoplasm. Therefore, these types of organelles can be inherited to the offspring and have a developmental continuity within the organism. The metabolism and thus the structure of plastids vary along with the differentiation of the organs, tissues and specific cells of the plant body. This fluctuation can continue during the entire life cycle as part of the developmental program of the host cell that harbours them. However, plastid differentiation is also strongly influenced by changes in the environmental conditions.

2.5.1. Proplastid

Plastids are found in high number in plant cells. There are many types in the plant cell. Chromoplasts are pigment-containing plastids, while colourless plastids are called leucoplasts. These plastids can be changed from one to other. Originally, they develop from proplastids. Proplastids are colourless bodies in meristematic or immature cells.

2.5.2. Etioplasts

Etioplasts, another type of plastids, are found in stems, but not in roots. They are a long-lasting intermediate stage in the way of differentiation from proplastid to chloroplast when there is very low intensity of light or it is dark. Etioplasts restart the differentiation toward chloroplasts as soon as the light is intense enough.

2.5.3. Leucoplasts

Leucoplasts are colourless plastids (without pigments), that function as storage organelles. Leucoplasts comprise amyloplasts, elaioplasts (or oleoplasts), and proteinoplasts. They store starch, lipids and proteins, respectively.

Amyloplast is an organelle in plants that stores starch. Amyloplasts are often found in nonphotosynthetic tissue, such as roots and storage tubers. In plant cells, amyloplasts synthesize starch, and all the stored starch in a cell can be found in these organelles as starch granules. Amyloplasts, apart from storing starch, are gravity sensors in root cells. Starch granules are denser than water so that amyloplasts fall to the bottom of the cell. These organelles interact with microtubules of the meristematic cells in a way that during cell mitosis the division plane is perpendicular to the gravity vector (which points to the Earth centre). Amyloplasts are also involved in the metabolism of nitrogen.

Elaioplasts are a type of leucoplasts specialized for lipid storage in plant cells. They have been demonstrated to be involved in the biosynthesis of terpenes in essential oil, which are then exported into the secretory pocket, greatly affecting the aroma and the taste of fruit in citrus species which means the importance of these organelles. Elaioplasts are small size plastids containing oil and lipids in fat drops. In plant cells, there are two synthetic pathways for lipids. The eukaryotic pathway depends on the smooth endoplasmic reticulum, whereas the so-

called prokaryotic pathway involves plastids. These two pathways produce different types of lipids. Elaioplast are also involved in pollen maturation. Some plants are able to store lipids in other organelles known as elaiosomes, which are derived from endoplasmic reticulum.

Proteinoplasts contain a high concentration of proteins, so high that proteins form crystals or very dense amorphous material. However, it is not clear yet if there is a type of plastids dedicated exclusively to protein storage in plant cells.

2.5.4. Chromoplasts

Chromoplasts contain carotenoid pigments that give the red, orange and yellow colours to the plant structure where they are present. These plastids are abundant in flowers, fruits, old leaves, and some roots. It is thought that one of their main functions is to attract animals for pollination or for spreading the seeds. Chromoplasts are metabolically active, though they contain fewer DNA copies than chloroplasts.

Chromoplasts have lipid drops containing carotenoids and complex molecules known as fibrils, which also contain a core with carotenoids. Chromoplasts are differentiated from chloroplasts, as well as from proplastids. During differentiation from chloroplasts, the photosynthetic machinery, mostly in thylakoids, is degraded and carotenoids are synthesized along with the compartments where they are going to be included. These compartments, known as plastoglobules, are lipid drops made up of mainly triacylglycerides that are located in the stroma. Carotenoids, mainly xanthophylls, are stored inside the plastoglobules, so concentrated that they can form filaments or crystals. Plastoglobules may be also found in other plastids. Chromoplasts develop an internal membrane system in the outer part of the stroma. These new membranes arise from the inner membranes and do not from the old degraded thylakoids. Carotenoids like lutein, beta-carotenoid, and others, may be also associated to these new membranes. Occasionally, the internal membranes show a reticular arrangement. Chromoplasts are non-photosynthetic plastids that accumulate carotenoids. They derive from other plastid forms, mostly chloroplasts. The entire set of Calvin cycle and of the oxidative pentose phosphate pathway persist after the transition from chloroplast to chromoplast.

2.5.5. Gerontoplasts

Chlorophyll and its degradation products are dangerous and lethal for cells because of their photoreactive features. Chloroplasts can be transformed to gerontoplasts, which resemble to chromoplasts. In the gerontoplasts, grana are unstacked, thylakoid membranes are lost and lipid-like plastoglobuli can accumulate. The efficiency of photochemical reactions and Calvin-Benson cycle is declined. There is a possibility the gerontoplast to transform back chloroplast till the terminal phase of cell death.

2.5.6. Chloroplasts

Chloroplast is found in any chlorophyll-containing plastids which are in plants and algal cells with photosynthesis. These lens-shaped organelles are bounded by a double membrane (Fig. 2.2.). Thylakoids are membranous structures in chloroplasts and they can pile up into stacks called granum. These compartments are surrounded by a gel-like matrix, its name is stroma. Photosynthesis has two types of reactions. The light-dependent reactions of it can occur on

the thylakoid membranes and those reactions which are independent from light can take place in the stroma.



Guard cells contain chloroplasts but epidermis cells not.

Fig.2.2. Structure of chloroplast in plant cell.

(Source: Wikimedia Commons. Author: Kelvinsong. This file is licensed under the Creative Commons Attribution 3.0 Unported license. Link to file: <u>https://commons.wikimedia.org/wiki/File:Chloroplast_II.svg</u>. Link to licence: https://creativecommons.org/licenses/by/3.0/deed.en)

2.6. Plant Cytoskeleton

The cytoskeleton organizes the cell and helps traffic organelles.

The plant cytoskeleton has two important components: microtubules and microfilaments. Each type is filamentous, with fix diameter and variable lengths, some micrometers. They are assemblies of globular proteins.

Microtubules has a diameter of 25 nm, they formed from polymers of the protein tubulin. The tubulin monomers are heterodimer composed of two polypeptide chains (α and β tubulin). Hundreds or thousands of tubulin monomers can arrange a column called protofilaments.

Microfilaments have a dimeter of 7 nm and they build up from globular actin, called G-actin. These monomers can be polymerized in order to form protofilaments. In the polymerized protofilaments, the actin has a name, F-actin. One microfilament contains two actin protofilaments in a double helical pattern.

Assembly Events of Microtubules and Microfilaments

Actin and tubulin subunits can exist as a pool of free proteins and their polymerized forms. Every monomer contains a bound nucleotide, ATP in the actin and GTP in the case of tubulin. Microtubules and microfilaments are polarized, so the ends are different. Microfilaments and microtubules have half-lives, some minutes, and determined by accessory proteins, actinbinding proteins (ABPs) in microfilaments and microtubule-associated proteins (MAPs) in microtubules.

Cortical microtubules are moving around the cell by "tread milling". Microtubules in the cortical cytoplasm migrate laterally around the cell periphery by a process called tread milling. During this process, tubulin heterodimers are added to the growing end at the same rate as tubulin is being lost from the other end, it seems like microtubules can move through the cytoplasm. Microtubules can play an important role in the polarity of the plant.

Cytoplasmic streaming and organelle trafficking

Molecular motors can help the movements of organelles through the cytoplasm. These motor proteins have a similar structure. They consist of a large globular head, a neck region and a tail which can connect to the cargo-binding domain. The head has the microtubule/actinbinding and the motor domains. The motor domain has ATPase activity; ATP hydrolysis provides the energy for the unidirectional movement via ATP-dependent cyclic conformation changes in the cytoskeleton-binding head. Molecular motors differ from each other considering the cytoskeletal elements they bind and the direction of their movement. Myosins move along the actin microfilaments toward the plus end of the F-actin. Kinesins can move along the microtubules, however, two-third of them are moving toward to the plus ends, while one-third of toward the minus end. Dyneins are absent from plant cells; in animal cells they are negative end motors of the microtubules.

Cytoplasmic streaming is a coordinated movement of particles and organelles through the cytosol, and occurs in all plant cells, but with variable velocities. One example of the developmentally regulated cytoplasmic streaming is the migration of the nucleus to the site of root hair formation in the root epidermal cells. However, environmental conditions also can induce cytoplasmic streaming, e.g. chloroplasts can be repositioned by light in the leaf cells in order to maximize or reduce exposure of light.

Summary

- 1. Plant cell walls are typical plant cell structures.
- 2. Plant cells contain compartments derived from the endomembrane system.
- 3. Chloroplasts and mitochondria are not derived from the endomembrane system. The endomembrane system plays a central role in secretory processes, membrane recycling, and the cell cycle.

- 4. The composition and fluid-mosaic structure of the plasma membrane permits regulation of transport into and out of the cell.
- 5. The endomembrane system conveys both membrane and cargo proteins to diverse organelles.
- 6. The specialized membranes of the nuclear envelope are derived from the endoplasmic reticulum (ER), a component of the endomembrane system.
- 7. The nucleus is the site of storage, replication, and transcription of the chromatin, as well as being the site for the synthesis of ribosomes.
- 8. The ER is a system of membrane-bound tubules that form a complex and dynamic structure.
- 9. The rough ER (ER) is involved in synthesis of proteins that enter the lumen of the ER, the smooth ER is the site of lipid biosynthesis.
- 10. The ER provides the membrane and internal cargo for the other compartments of the endomembrane system.
- 11. Secretion of proteins from cells begins with the RER.
- 12. Glycoproteins and polysaccharides destined for secretion are processed in the Golgi apparatus.
- 13. During endocytosis, membrane is removed from the plasma membrane by formation of small chlatrin-coated vesicles.
- 14. Endocytosis from the plasma membrane provides membrane recycling.
- 15. Oil bodies, peroxisomes, and glyoxysomes grow and proliferate independently of the endomembrane system.
- 16. Mitochondria and chloroplasts each have an inner and an outer membrane.
- 17. Plastids may contain high concentrations of pigments or starch
- 18. Proplastids pass through distinct developmental stages to form specialized plastids.
- 19. In plastids and mitochondria, DNA replication and fission are regulated independently of nuclear division.
- 20. A three-dimensional network of microtubules and microfilaments organizes the cytosol.
- 21. Microtubules and microfilaments can assemble and disassemble.
- 22. Molecular motors associated with components of the cytoskeleton move organelles thorough the cytoplasm.
- 23. During cytoplasmic streaming, interaction of the F-actin with myosin provides for independent movement of organelles, including chloroplasts.
- 24. The cell cycle, during which cells replicate their DNA and reproduce themselves, consists of four phases.
- 25. Successful mitosis and cytokinesis require the participation of microtubules and the endomembrane system.
- 26. Tubular extensions of the plasma membrane traverse the cell wall and connect the cytoplasm of clonally derived cells, allowing water and small molecules to move between cells without crossing a membrane.

Review Questions

- 1. What are the typical plant cell organelles?
- 2. Which organelles belong to the endomembrane system in plant cells?
- 3. Which organelles are independently dividing and derived from the endomembrane system?
- 4. Which organelles are independently dividing and semiautonomous?
- 5. How is the plant cytoskeleton organized?
- 6. What is the factors of cell cycle regulation?
- 7. What are plasmodesmata in plant cells?

Discussion Questions

- 1. What are the differences in different types of plant cell walls?
- 2. What types of cell walls can be found in the plant body?
- 3. How do Golgi body and ER affect lipid and carbohydrate biosynthesis in plants?
- 4. Why are plastids so important in plant cells?

Additional Reading

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Chapter 3. Mineral nutrition

This chapter introduces the plant mineral nutrition, with a discussion on the importance of mineral assimilation. This is followed by a discussion of the main groups of minerals in plant matter. The chapter explores the main rules of nutrient uptake efficiency and the pH dependence of it. The main nutrient deficiencies are briefly surveyed.

Learning goals

> Knowledge:

- The students know to classify the nutrients according to their relative amount in plants.
- The students know to classify the nutrients according to their function in plants.
- The students understand the main law determining plant nutrition.
- The students can define cation exchange capacity
- The students can explain how soil pH influences the nutrient uptake of plants.
- The students can distinguish among the mobile and immobile elements in plants.
- > Abilities:
- The students can use the obtained knowledge to investigate appropriate soil parameters to estimate the efficiency of nutrient uptake by plants
- The students can recognise deficiency symptoms and link those to the appropriate macronutrients
- > Attitude:
- The students are open to study the effect of environmental parameters on plant nutrition
- Autonomy/responsibility
- The students can autonomously determine appropriate conditions to grow a plant
- The students feel responsibility to provide the best possible conditions for their plants

Inorganic substrates called minerals required by our body for a variety of different functions thus influence our health, mood and our life. For instance, minerals are involved in the formation of bones and teeth; they are components of enzyme systems and they are involved in normal nerve function. Our optimal mineral nutrition requires attention and is a crucial point in human feeding. The nutrients in the matter that makes up all animals and humans originated from plants mineral nutrient assimilation. As plants are in the bottom of the food chain, minerals enter the biosphere predominantly through the root systems of plants. For their unique role in mineral uptake plants are called "miners" of Earth's crust (Epstein 1999, Taiz and Zeiger 2014). The large surface area of plant roots and the ability to absorb ions at low concentrations from the soil solution make mineral absorption a very effective process.

3.1. Nutrients

Plants are mostly **autotrophic** organisms, so they produce complex organic compounds from simple substances. These simple substrates are inorganic components present in the plants surroundings: plants uptake carbon dioxide from the atmosphere and water and mineral

nutrients from the soil. **Heterotrophic** organisms depend on energy-rich organic molecules previously synthesized by other organisms.

Plant nutrition is often separated into two topics: **organic nutrition and inorganic nutrition.** The production of carbon compounds is regarded as organic nutrition, specifically the incorporation of carbon, hydrogen, and oxygen via photosynthesis, while inorganic nutrition is focused primarily on the acquisition of mineral elements from the soil.

According to their average amounts in plant organisms and their importance, elements can be divided into four groups: **organogenic elements, macronutrients, micronutrients and potentially beneficial elements** (Table. X.1).

Certain elements have been determined to be **essential** for plants. An **essential element** is defined as one that is intrinsic **component in the structure or metabolism**, and whose **absence causes several abnormalities** in plant growth, development, or reproduction, thus prevents a plant from completing its life cycle (Arnon and Stout 1939).

Out of the four groups (organogenic elements, macronutrients, micronutrients and potentially beneficial elements), macro- and micronutrients are regarded as essential elements. If plants are given these essential elements, water and energy from sunlight, they can synthesize all the compounds required for normal growth. Organogenic elements such as hydrogen, carbon, and oxygen are not considered mineral nutrients because they are obtained primarily from water or carbon dioxide.

From a physiological viewpoint it is difficult to justify the classification of plant nutrients according to their amounts in plants. Besides the classification of essential elements into macro and micro nutrients Mergel and Kirkby (1978) proposed a classification according to the biochemical role and physiological function of the elements. Elements can be divided into four main groups regarding their functions and roles:

The first group contains major constituents of plant material: carbon, hydrogen, oxygen, nitrogen and sulphur. Plants assimilate these nutrients via biochemical reactions involving oxidation and reduction.

The second group contains phosphorus, boron and silicon, which are important in energy storage reactions or in maintaining structural integrity. Elements in this group are often present in plant tissues as phosphate-, borate-, and silicate-esters.

3.2. Liebig's law of the minimum and Mitscherlich's law of diminishing returns

The relationship between plant production and nutrient levels is crucial for agriculture, since this knowledge enables the monitoring of current fertility programmes, the development of corrective strategies for the soil and economic use of resources, thus helping to achieve productive crop plants (Ferriera 2017).

Some basic laws and mathematical functions are generally used to describe the theoretical relationship between plant production and fertilizer doses.

The principle of Liebig's law or the law of the minimum in agricultural science was developed by Carl Sprengel (1828) and later it was popularized by Justus von Liebig. The Liebig's Law of the Minimum postulates that **plant's growth is limited by the nutrient in shortest supply**. According to this law, the limiting element is the one that has the least relative quantity in the plant or soil and, for this reason; it controls growth (Paris 1992, Ferriera 2017). In other words, it states that not the total amount of resources available controls the plant growth, but it's controlled by the scarcest resource (limiting factor). The first attempt to interpret the law of minimum with a functional specification is attributed to the German agronomist Alfred Micherlich, who in 1909 published a study showing that crop response in likely to follow an exponential specification (Chesworth 2016; Fig. 3.2). The classical Mitscherlich equation is based on Liebig's Law of the Minimum and **describes how an increase in the main factor that is limiting growth influences the yield of a crop** (Fig. 3.1).

Source		Element	Chemical symbol
Obtained from water or carbon dioxide	Organogenic elements components of organic molecules	Hydrogen	Н
		Carbon	С
		Oxygen	0
Obtained from the soil	Macronutrient s up to 1.5%, 15,000 ppm in dry matter	Nitrogen	Ν
		Potassium	Κ
		Calcium	Ca
		Magnesium	Mg
		Phosphorus	Р
		Sulphur	S
	Micronutrient	Iron	Fe
	100 ppin (dry matter) or less	Manganese	Mn
		Boron	Ro
		Copper	Cu
		Zinc	Zn
		Molvbdenum	Mo
		Chlorine	Cl
		Cobalt	Со
		Nickel	Ni
	Potentially beneficial elements (not essential)	Sodium	Na
		Vanadium	V
		Aluminium	Al
		Silicon	Si

Table. 3.1. Classification of plant mineral nutrients according to concentration in dry matter

The third group is made up of potassium, sodium, calcium, magnesium manganese and chlorine. These elements present in plant tissue as either free ions dissolved in the plant water or ions electrostatically bound to substances such as the pectic acids. Members of this group have roles as enzyme cofactors and in the regulation of osmotic potentials.

The fourth group comprises ion copper, zinc and molybdenum. These elements predominantly present as chelates in the plant, and have important roles in reactions involving electron transfer.

3.3. Mineral content of plant material

The material of green plants consists of organic matter, water and minerals. The relative amounts of these three materials may vary but in living plants, water is always in the highest proportion, while minerals in the lowest (Mengel and Kirkby 1978). Average percentage distribution is the following: 70% water; 27% organic material; 3% minerals.

The main factor controlling mineral content of plants is a genetically determined uptake potential for each mineral nutrient. Besides this potential another important factor is the availability of plant nutrients in the nutrient medium or soil.



Fig. 3.1. Mitscherlich's law in terms of nutrient application (Figure of Á. Gallé)

3.4. Soil

The physically, chemically, and biologically complex matter of soil is quite heterogeneous substance, which contains solid, liquid, and gaseous phases. All of these phases interact with minerals. In the solid phase, the inorganic particles provide a reservoir of potassium, calcium, magnesium, manganese, sodium and iron, while organic compounds contain nitrogen, phosphorus, and sulphur, among other elements. The liquid phase of soil is called soil solution, and contains dissolved mineral ions and serves as the medium for ion movement to the root surface.



Fig. 3.2. Ion distribution with increasing distance from the charged surface (according to Gouy-Chapman-model) (Figure of Á. Gallé).

Although oxygen, carbon dioxide, and nitrogen gases are dissolved in the soil solution; roots exchange gases with soils predominantly through the air gaps between soil particles.

Colloidal (organic or inorganic) soil particles are predominantly negatively charged, which attract cations such as Ca^{2+} , Mg^{2+} , K^+ , Al^{3+} and Mn^{2+} . With increasing distance from the colloid surface the cation concentration decreases (rapidly at first), and the anion concentration increases in a reciprocal way (Fig. 3.2). The mentioned strongly attracted cations are adsorped to soil particles and are not easyly lost when the soil is leached by water. These cations provide a nutrient reserve available to plant roots, which is an important factor in soil fertility.

3.5. PH dependence of nutrient uptake

The proton concentration in soils is rather low and affects the growth of plant roots and microorganism. As H^+ ions can be absorbed to soil colloid one may distinguish between the actual pH, which is the H^+ concentration of soil solution and potential pH, which includes H^+ ions absorbed to the soil colloids.

The pH of soil solution has a strong effect on soil constituents, especially on minerals, microorganisms and plant roots. Acidity of soil solution causes the weathering of rocks, which releases various ions for instance K^+ , Mg^{2+} , Ca^{2+} . Solubility of salts including carbonates, phosphates and sulphates is higher also in lower pH.

Optimal pH for crop growth is influenced by the soil texture, for instance it is rather acid in organic soils and for mineral soils it rises with increasing clay content. Thought pH values of soils may differ widely (3 to 10), plant roots generally prefer acidic soils, at pH value between 5.5 and 6.5. H⁺ concentration of soil solutes influences the plant nutrient uptake. On Figure 3.4, the effect of pH on nutrient availability is shown in organic soil. To a varying degree plants are able to tolerate and to cope with differences in pH of soil solution, and with the effects of pH induced changes. Thus the pH optimum for individual crops often differ, for instance wheat prefer pH 4.1-7.4 range, while for oilseed rape pH 6.0-7.5, and for potato pH 5.5-6.5 is optimal.

3.6. Nutrient deficiency

Plant nutrition disorders or deficiency symptoms are caused by inadequate supply of an essential element. The easiest way to investigate the symptoms is to grow plants in **hydroponic** system, in nutrient solution. For growing plants in solution culture (hydroponics) it is necessary to avoid unexpected changes in the nutrient concentration, therefore requires large volume of nutrient solution or frequent adjustment of the components and pH.

The most commonly used and most comprehensive nutrient solution is called Hoagland solution, which was named after Dennis R. Hoagland researcher, who firstly formulated it. A version of this nutrient solution is called modified Hoagland solution, which contains all mineral elements needed for fast plant growth (Table. 3.2). The concentrations of the elements are set to the highest possible levels in this solution, without causing toxic symptoms.


Fig. 3.3. Soil particles and ions in two soil differing in cation exchange capacity. (Figure of Á. Gallé)

	N			
	P			
	K			
	N			
	5			
	Ca			
	Mg			
	Fe			
	Mn			
	B			
	Cu			
	Zn		_	
	Mo			
	P	lctinomy	cetales	
		Fungi		
1				

pH 4.0 5.0 6.0 7.0 8.0 9.0 10.0

Fig. 3.4: PH dependence of nutrient availability in organic soils. Wider shaded areas indicate the higher availability. (Figure of Á. Gallé)

The easiest way to investigate the deficiency symptoms is withholding of an essential element from the nutrient solution of hydroponics. These symptoms are regarded as acute symptoms. Mineral elements are having multiple roles in plants, thus can participate in several physiological reactions. Some general function is connected to structure, metabolism and osmoregulation. Regarding to deficiency symptoms an important clue is the facility of plant to re-utilise the element. If an element can be transported from the old leaves to the young leaves and re-utilised, nutrient is so called **mobile**. Nitrogen, potassium, magnesium, phosphorus, sodium, chloride, zinc and molybdenum are mobile nutrients, while calcium, sulphur, iron, boron and copper are **immobile**. In the case of mobile nutrients, the symptoms appear on the older leaves, while the deficiency of immobile elements cause visible symptoms on the younger leaves. Further the specific nutrient deficiencies are described.

Component	Stock solution $g L^{-1}$	Volume (mL) of stock solution per liter of final solution				
Macronutrients						
KNO ₃	101.1	6.0				
$Ca(NO_3)_2 \bullet 4H_2O$	236.16	4.0				
NH ₄ H ₂ PO ₄	115.08	2.0				
MgSO ₄ •7H ₂ O	246.49	1.0				
Micronutrients (from one stock solution)						
KCl	74.55	2.0				
H ₃ BO ₃	61.83					
MnSO ₄ •H ₂ O	169.01					
$ZnSO_4 \bullet 7H_2O$	287.54					
CuSO ₄ •5H ₂ O	549.68					
H ₂ MoO ₄	161.97					
NaFeDTPA	468.20	0.3-1.0				

Table 3.2. Composition of a modified Hoagland solution for growing plants.



Fig. 3.5. Nutrient deficiency symptoms. (Figure of Á. Gallé)

3.7. The most important elements for plants

3.7.1. Nitrogen

Nitrogen is one of the most widely distributed element in Earth, largest nitrogen amounts present in fixed forms in the earth's crust. Atmosphere is the second largest reservoir of nitrogen, and is circulating between air, soil and living organisms. In plants and other organisms, it is a constituent of several cell components, such as amino acids, nucleic acids. Inorganic molecular form of nitrogen can be converted to an organic form in a process called nitrogen fixation. This process is provided by free living (e.g. *Azotobacter, Clostridium, Pseudomonas*) and symbiotic (e.g. Rhizobium) microorganisms. In plant nitrogen is being converted from inorganic to organic forms. For this the most important inorganic sources are NO₃⁻ and NH₄⁺, which can be taken up and metabolised by plants.

As nitrogen is a mineral, which plants required in the greatest amounts, its deficiency is characterised by poor growth rate. In most cases nitrogen deficient plant's leaves are turning yellow, which symptom is called **chlorosis**. As nitrogen is a mobile nutrient, the chlorosis appears on the oldest leaves near to the base of the plant, while the rest of the plant is often light green. Slowly developed nitrogen deficiency can cause slender and often woody stems, and in some plants (tomato, and some corn varieties) the excess carbohydrates, which were not used in nitrogen metabolism are converted to anthocyanins, resulting in a purple colouration of leaves. Lack of nitrogen often cases outstanding elongation of the roots compared to a healthy plant.

3.7.2. Sulphur

In soil, the organically bound sulphur provides larger S reservoir than the inorganic forms of the element. Microorganisms provide available sulphur for plants form the organic sulphur fraction. The process depends on the media conditions: under aerobic conditions the formed H_2S undergoes autoxidation to SO_4^{2-} , while in anaerobic media H_2S can be oxidised to elemental sulphur by chemotrophic sulphur bacteria. Under aerobic condition, the oxidation of sulphur can result in the formation of H_2SO_4 , thus soil pH can consequently decrease. Similarly, the addition of elemental sulphur to soils results in acidification, which can be used a beneficial treatment of alkaline soils.

As mentioned above, aerobic soil conditions promote SO_4^{2-} formation, which is the form of sulphur plants mainly absorb. In plant cells, sulphur is a component of two amino acids (methionine and cysteine), several coenzymes and vitamins. Sulphur deficiency result in an inhibition in protein synthesis. Lack of sulphur causes similar symptoms as nitrogen deficiency, with one big difference: plant cannot mobilise this element, thus chlorosis will be visible firstly on young leaves.

3.7.3. Phosphorus

Phosphorus in soil occurs mostly as phosphates (PO_4). Large amount of the phosphates is associated with soil organic matter. According to plant nutrition and availability of phosphorus, there are three important main phosphate fractions: in the soil solution, in the labile pool and in the non-labile pool. In labile fraction the soil phosphate is in rapid pH dependent equilibrium with soil solution. In the third fraction phosphate is insoluble, therefore can only be released into labile pool. Plant roots contact with phosphate in soil solution. As roots have high demand for phosphorus, which creates a concentration gradient between the soil near the root surface and bulk soil; and regulate the phosphate diffusion towards the roots. The concentration of phosphate in plant roots is about 100 to 1000 higher than in the soil solution.

Phosphorus is important component of several compounds, for instance phospholipids that makes up all membranes and nucleic acids and nucleotides such as ATP. The deficiency of this element results in decreased growth gathered with deep greenish or purple coloration leaves. Sometimes on dark green leaves **necrotic spots** (dead tissue spots) appear.

3.7.4. Potassium

The greatest part of potassium in soil is trapped in the clay minerals, therefore is hardly leached out by cation exchange. For this reason, clay rich soil usually rich in potassium also. Potassium is highly mobile in the soil, and clay content considerably influences the movements of it in soil. Plants can uptake potassium from the soil solution.

Potassium is the most important cation in plant physiology. Besides its content in plant tissues potassium fulfils important physiological and biochemical roles. As plants are able to mobilise potassium deficiency symptoms firstly appear on more mature leaves. The first symptom of potassium deficiency is chlorosis developing from the tips and margins of the leaves. Later the chlorotic regions turn into necrotic lesions. These leaves can curl up and wither. Stems of potassium-deficient plants are weak, with short intermodal region as formation of xylem and phloem tissues are restricted and vascular bundles are lignified. These plants are often more susceptible to diseases and to damages caused by inappropriate environmental conditions such as drought.

3.7.5. Calcium

Calcium in soil present in primary minerals (such as Ca phosphates, Ca carbonates and Ca baring Al-Si-silicates), and Ca^{2+} is absorbed to organic and inorganic soil colloids. These ions promote the coagulation of soil colloids and improve soil structure. In crop production, the calcium deficiency is not very common as most inorganic soil contain high enough levels of in soil solution Ca^{2+} . In some acid peat soils, where natural Ca content can be so low, that using calcium containing fertilisers is reasonable.

Calcium fulfils various functions in plants: it is used in the synthesis of cell walls, in the mitotic spindle during cell division, in cell membranes, and as a secondary messenger. Deficiency symptoms of calcium are characteristic and the most severe of all. In the absence of calcium young meristematic regions (tip of young roots and leaves) necrotize. These symptoms can be preceded by chlorosis in slowly grooving plants. Roots system is also affected by lack of calcium, roots became brownish, highly branched and short. In horticultural plant production plants can suffer from relative calcium deficiency, when the Ca content is appropriate in the soil and plants (for instance pepper or tomato) are having difficulties with mineral uptake. It often occurs in warm green houses. To solve these problems calcium containing foliar fertilisers can be used.

3.7.6. Magnesium

Soil Mg content can be divided into exchangeable and non-exchangeable and water soluble forms, where the largest fraction of magnesium is in the non-exchangeable form. This nutrient similarly like calcium can easily be leached from the soil, but in many soils removal by leaching is in balanced with the release of Mg^{2+} by weathering.

In plants, magnesium is having roles in enzyme activations and photosynthesis (both as enzyme activator and as component of chlorophyll), and in DNA and RNA synthesis. The main symptom of magnesium deficiency is the interveinal chlorosis, which is firstly occurring on older leaves. These leaves can later turn yellow or white. Exposition of an Mg deficient plant to strong sunlight may cause withering of the whole plant. In some cases, abscission of premature leaves is also a characteristic of Mg deficiency.

3.7.7. Iron

Iron is the fourth most abundant element found in soil, by weight it makes up about 5% of the Earth's crust. In soil it mostly present in forms that cannot be taken up by plants. Compared with the total amount in soil, the soluble iron $(Fe^{3+}, Fe(OH)_2^+, Fe(OH)_2^+$ and $Fe^{2+})$ is in extremely small amounts. Iron is able to form organic complexes or chelates, which can facilitate the Fe movement in soil, thus movements towards the plant roots.

Iron often participates in electron transfer reactions as Fe²⁺ can reversibly oxidised to Fe³⁺. Characteristic symptom of iron deficiency is chlorosis of leaf veins similarly as magnesium deficiency, but in the case of iron the symptoms appear on the youngest leaves. If the lack of iron is prolonged, the whole leaves can turn light yellow or white.

Summary

- 1. As plants are in the bottom of the food chain, minerals enter the biosphere predominantly through plants. Elements can be divided into four groups according to their average amounts in plant organisms: organogenic elements, macronutrients, micronutrients and potentially beneficial elements.
- 2. Certain elements have been determined to be essential for plants. An essential element is defined as one that is intrinsic component in the structure or metabolism, and whose absence causes several abnormalities in plant growth, development, or reproduction, thus whose absence prevents a plant from completing its life cycle.
- 3. The relationship between plant production and nutrient levels is crucial for agriculture. The Liebig's Law of the Minimum postulates that plant's growth is limited by the nutrient in shortest supply, while Mitscherlich describes how an increase in the main factor that is limiting growth influences the yield of a crop.
- 4. Soil particles are predominantly negatively charged, which attract cations. The total capacity of a soil to hold exchangeable cations is called cation exchange capacity (CEC), which highly dependent on soil type.
- 5. The pH of soil solution has a strong effect on soil constituents, especially on minerals, microorganisms and plant roots, thus mineral uptake depends also on the H⁺ concentration of soil solution.
- 6. The easiest way to investigate the deficiency symptoms is withholding of an essential element from the nutrient solution of hydroponics. The most commonly used and most comprehensive nutrient solution used for hydroponics is called Hoagland solution.

- 7. If an element can be transported from the old leaves to the young leaves and re-utilise it, nutrient is so called mobile. Nitrogen, potassium, magnesium, phosphorus, sodium, chloride, zinc and molybdenum are mobile nutrients, while calcium, sulphur, iron, boron and copper are immobile.
- 8. Nitrogen deficiency is characterised by poor growth rate, and chlorotic leaves. As nitrogen is a mobile nutrient, the chlorosis appears on the oldest leaves near to the base of the plant, while the rest of the plant is often light green.
- 9. Lack of sulphur causes decreased growth rate and chlorosis on young leaves.
- 10. The deficiency of phosphorus results in decreased growth gathered with deep greenish or purple coloration leaves.
- 11. Symptoms of potassium deficiency are chlorosis and necrosis developing from the tips and margins of the leaves. Stems of potassium-deficient plants are weak.
- 12. In the absence of calcium young meristematic regions (tip of young roots and leaves) necrotize, and roots became brownish, highly branched and short.
- 13. The main symptom of magnesium deficiency is the interveinal chlorosis, which is firstly occurring on older leaves.
- 14. The lack of iron is causes light yellow or white colour of leaves.

Review questions

- 1. Why can plant nutrition be important for humans?
- 2. How can plant nutrient be classified? Why do some classifications differ? What is the theoretical basic of them?
- 3. What does essential element means?
- 4. What does Liebig's law of the minimum and Mitscherlich's law of diminishing returns claims?
- 5. If the cation exchange capacity of an agricultural land is higher than others, does it mean that it contains more available plant nutrient?
- 6. Can plants grow and complete a life cycle without soil?
- 7. List general (frequent) deficiency symptoms!

Discussion question

1. How can Liebig's law of the minimum and Mitscherlich's law of diminishing returns be used in agricultural production?

2. Why can applying the same fertilizer for years on the same field be problematic?

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Chapter 4. Short and long distance transport

This chapter introduces the plant transportation processes, beginning with a discussion on the chemical potential. The chapter explores the main rules of water uptake. The long distance transport through xylem and phloem is briefly surveyed. This is followed by a discussion of the main types of transmembrane proteins: pumps, carriers and channels.

Learning goals

> Knowledge:

- The students know the levels of plant transport.
- The students know the main forces behind water movement.
- The students understand the definition of water potential.
- The students know the two main ways of water movement in plant tissues.
- The students can explain the main forces behind water and solute movement in plants.
- The students can distinguish among the active and passive transport processes.
- The students can explain how pumps, carriers, channels work.
- > Abilities:
- The students can use the obtained knowledge to define optimal parameters for a plant to use water efficiently
- The students can recognise what is the problem if a plant has symptoms of disturbed water/solute transport
- > Attitude:
- The students are open to do experiments with plants to better understand their transport processes
- Autonomy/responsibility
- The students can autonomously determine appropriate conditions to grow a plant
- The students feel responsibility to provide the best possible conditions for their plants

Transport in plants occurs on three levels: on cellular level, tissue level (short distance transport) and whole plant (long distance transport). Individual cells uptake and release of solutes and water, root cells absorb water and minerals from the soil. Short-distance transport is when substances are transported from cell to cell for example loading of sucrose from photosynthetic cells into the sieve tube cells of the phloem. A whole plant phenomenon is long-distance transport e.g. transportation of sap within the xylem and phloem or transport of photosynthete from leaf to root.

In plants, the largest flow of water is from soil, through plants to the atmosphere. In nature, liquid water originates from rain and enters the system following rainfall. Survival of the plant depends on balancing water uptake and water loss.

4.1. Chemical potential of water

For understanding the movement of water in plants, let us suppose water moves spontaneously from plant to atmosphere. In this case the free energy (Gibbs free energy, G) of the water must decrease. This free energy per mole of substance is termed the chemical potential (μ). It is a quantitative expression of the free energy associated with a substance. Chemical potential of

the water represents the free energy associated with water, which determines the direction of spontaneous water flow: it will move from high to low chemical potential. The bigger difference in chemical potential results in greater driving force for water flux.

The chemical potential of water at constant temperature depends on its activity (a_w) , pressure (P) and height (h) in a gravitational field. This is expressed with formula as:

$$\mu_w = \mu_w^0 + RT \ln a_w + \overline{V}_w P + m_w gh$$

 $\mu_w^{\ 0}$ – arbitrary chemical potential of water (w) under standard circumstances

- R universal gas constant (8,314 J mol⁻¹ K⁻¹)
- T absolute temperature (K)
- a_w activity of water
- Vw-water's partial molar volume
- P-pressure
- m_w mass per mole of water
- g acceleration due to gravity
- h height in a gravitational field

The chemical potential of water (μ_w) under standard circumstances (μ_w^0) is pure water at atmospheric pressure and the height (and temperature) of the system under consideration. The unit of the chemical potential is: J mol⁻¹.

4.2. Water potential and water potential gradients

The formula of water chemical potential can be transformed to a more practical form, firstly by defining 'water potential' as **the difference between the chemical potential of water at any point in a system and that of pure free water in a standard state**. Water potential is abbreviated with the Greek letter psi: Ψ_w . The concept of water potential was introduced in 1960 by R.O. Slatyer and S.A. Taylor. Ψ_w measures the free energy of water per unit volume (J m-3), which units are equivalent to pressure units such as the pascal. **The commonly used measurement unit for water potential is pascal.**

$$\Psi_{\rm w} = \frac{\mu_{\rm w} - \mu_{\rm w}^0}{V_{\rm w}}$$

The major factors influencing the water potential in plants are concentration, pressure and gravity, thus the water potential of solutions may be dissected into individual components. Water potential is expressed with the following formula:

$$\Psi_{\rm w} = \Psi_{\pi} + \Psi_{\rm P} + \Psi_{\rm g}$$

The terms Ψ_{π} and Ψ_{P} and Ψ_{g} symbolise the effects of solutes, pressure and gravity on the free energy of water.

 Ψ_P , the pressure potential may be positive, zero or, where water is under tension, negative; Ψ_{π} , the osmotic potential because of the presence of solutes and is always negative; Ψ_g the gravity potential, which is negligible in cells and small plants but may be significant in tall trees.

Osmotic potential or solute potential represents the effect of dissolved solutes on $\Psi_{w.}$ This is primary an entropy effect, dissolved solutes lower the free energy of the system. Osmotic potential can be estimated by the van't Hoff equation, where R is the universal gas constant (8,314 J mol⁻¹ K⁻¹), T is the absolute temperature (K) and c is the concentration of 's' solute.

$$\Psi_{\rm w} = -RTc_{\rm s}$$

Pressure potential Ψ_P or hydrostatic pressure can increase or decrease the water potential, as it can be positive or negative. Within cells positive hydrostatic pressure is called **turgor pressure**.

The gravity component of water potential, Ψ_g , depends on the height (h) of the water (above the reference-state water), the density of water (ρ_w) and the accelerating due to gravity (g).

$$\Psi_{\rm w} = \rho_{\rm w} {\rm gh}$$

On cellular level Ψ g is a negligible component compared to Ψ_P and Ψ_{π} . In these cases, water potential can be calculated by the following formula:

$$\Psi_{\rm w} = \Psi_{\rm P} + \Psi_{\pi}$$

Plant growth, biomass production, photosynthesis and the yield are all influenced by the water potential, as it determines the direction of water movement. Water only moves in response to water potential difference. Therefore, for water to move through the soil-plant-atmosphere system (transpiration), the Ψ_w should be as follows:

$$\Psi_{soil} > \Psi_{root} > \Psi_{stem} > \Psi_{leaf} > \Psi_{atmosphere}$$

	Water potential (MPa)
Soil	02.0
Root	~ -0.2
Shoot	~-0.3 1.0
Atmosphere 50% relative humidity	-93.6
Table 4.1 Water potential in soil	root and atmosphere

Table.4.1. Water potential in soil, root and atmosphere

The water potential in the soil ranges from close to zero in wet soil to rather negative values in dry soils and an atmosphere with 50% relative humidity is -93.6 MPa (Table 4.1) This large difference in free energy is the major driving force for water movement through the soil-plant-atmosphere system.

4.3. Water movement within plant body

Plant roots and leaves provide a number of possible pathways for the radial and longitudinal movement of water. The majority of radial movement occurs in those regions where root hairs are prevalent, and the direction of water movement is from the soil to the central vascular tissue. There are two main ways of water movement in tissues: **apoplastic** or **symplastic** transport. Water can flow through the cell walls (apoplastic transport or movement) or within cells (a

symplastic route where water flows from cell to cell via plasmodesmata) or a combination of the two (Fig. 4.1.).



Fig. 4.1. Apoplastic and symplastic transportation (Figure of Á. Gallé)

The apoplastic transport may hold danger as different solutes (potentially toxic or dangerous) could be carried by water into tissues without any regulation by living cells. To control these movements, plant roots have developed barriers to the radial movement of solutes. These are called Casparian bands, which contain insoluble suberin. These barriers present in the walls of specialised cells that make up the exodermis and the endodermis and force the symplastic transport of water and solutes. Their supposed function is to prevent unwanted flow of water and solutes and prevent the relatively concentrated solution in the xylem leaking from the root to the soil.

The **xylem** represents an apoplastic pathway. As there is relatively higher pressure in the roots and relatively lower pressure in the leaves it allows water to flows towards the leaves. The sap of xylem rises without the help of any mechanical pump, against gravity, sometimes reaching more than 100 m heights (tallest trees). As mentioned above water potential gradient determine the direction of water. There are three main forces behind the water and solutes movement in the xylem: transpiration is a major component forcing xylem transport during the day and is responsible for the negative pressure in xylem vessels. In the dark, when transpiration is low another gradient in the hydrostatic pressure (often called root pressure) forces water and solutes transportation in the xylem. It is created by the secretion of ions into the xylem, which results in osmosis and a consequent water flow through surrounding cells.

Besides this, water has adhesive and cohesive properties, which creates a tension (pulling) on the water in the xylem and pulls the water toward the direction of water loss. The hydrogen bonds provide the tensile strength of water. Even under a tension of several MPa water column does not break. The tension created by the evaporation of water together with the cohesive and adhesive forces (and hydrogen bonds) is enough to support this column against the forces of gravity.

The transportation in the phloem, represents a symplastic pathway. Water flow is pressure driven, but the pressures are positive in this case. There are relatively high pressure values in the source regions and relatively lower pressure values at the sinks. The xylem (except for root

pressure exudation) operates under negative hydrostatic pressure (tension), while the phloem is under positive hydrostatic pressure.

4.4. Chemical potential of solutes

Chemical potential could be defined as the potential energy resulting from the difference in concentration between the two compartments.

Besides water, chemical potential determines the direction of other solutes' movements in plants body (e.g. the movement of a charged ion across the membrane). If it is a charged solute, the potential has two components: the chemical (concentration) and the electrical potentials, thus, it is named electrochemical potential. Electrochemical potential is expressed with formula as:

 $\mu_i = \mu_i^0 + RT \ln a_i + \overline{V_i}P + z_iFE$

- μ_j Chemical potential of j material or solute
- μ_j^0 Chemical potential of j under standard circumstances
- R universal gas constant (8,314 J mol⁻¹ K⁻¹)
- T absolute temperature(K)
- a activity, without charge: C_j molar concentration
- $V_j j$ material's partial molar volume
- P-hydrostatic pressure
- z electrostatic charge
- F- Faraday's constant (96500 coulomb / mol proton)
- E overall electric potential



Fig. 4.2. Passive and active ways of transportation through membrane. (Figure of A. Gallé)

4.5. Membrane transport: pumps, carriers, channels

The transportation process is passive, when the solute and water is moving in the direction of the lower (electro-) chemical potential (Fig. 4.2). Solutes can cross membranes by simple **diffusion** by dissolving in the oily interior of the membrane (e.g. O_2 , CO_2 , H_2O_2) or can cross the membrane via channels (e.g. ions). Ion channels are integral membrane proteins, which function as regulated pores with open and closed states. Ion channels are purely passive transporters, with the chance of regulation. In this case the transportation is called **facilitated diffusion through channels**. The opening and closing of them depend on different factors, there are voltage-gated and ligand-gated channels and a third group is formed by mechanosensitive channels.

Another way of passive transportation via **facilitated diffusion is mediated by carrier proteins**. Unlike channels, **carrier proteins** do not have pores that extend completely across the membrane (Ördög and Molnár, 2011). Carriers provide specialized transportation of ions or organic metabolites. The transportation via carriers begins with the binding of the substrate to a specific site of the carrier protein. Binding causes a conformational change in the protein, which results in releasing the substance to the solution on the other side of the membrane, where the substance dissociates from the carrier's binding site. As this process requires a conformational change in the protein the rate of transport by a carrier is slower than that through a channel. For example, NH⁴⁺ is transported into the cell via a carrier-type facilitated diffusion mechanism.

Unlike passive transport, **active transport** through the membrane requires a source of energy, such as ATP hydrolysis. The transmembrane proteins that carry out the **primary active transport** are called **pumps**. Pumps move solutes across a plasma membrane against their concentration gradient, in contrast to ion channels, where solutes (e.g. ions) go via passive transport. The transported molecule can be variable; some pump can carry large organic molecules (e.g. ABC transporters), but for other pumps, H^+ is the principal ion that is electrogenically pumped across the membrane. These enzymes convert energy from various sources, including ATP and other redox reactions, to potential energy stored in an electrochemical gradient. Energy originated form the electrochemical potential gradient can be used in secondary active transportation processes by proteins including ion carriers and ion channels, to drive vital cellular processes, such as ATP synthesis.

As it was described in the previous paragraphs, passive diffusion can be facilitated by carrier proteins. If one solute is transported according to the direction of electrochemical potential gradient it is called **uniport** (Fig. 4.3). Carriers can perform carrier-mediated cotransport, which is *secondary active transport and is driven* indirectly by pumps, as the energy originated form the electrochemical potential gradient is used for these processes. The gradient of electrochemical potential for H⁺ referred to as **proton motive force (PMF)**. Carrier-mediated secondary active transport can be divided to two types: **symport and antiport**. When two substances move in the same direction through the membrane the transport is called **symport**, and when the solutes move in the opposite direction, it is called **antiport** (Fig. 4.3).

Pumps such as P type ATPases, Vacuolar type H+ ATPases or V-PPases generate proton motive force (gradient of electrochemical potential for H+) between the two sides of both tonoplast and vacuolar membrane (Fig. 4.4). The proton motive force is the primary energy currency in plants: proton gradients produced by conversion of energy from high-energy chemical bonds and by the photosynthetic and respiratory electron transport chains. As protons are compartmentalised (pumped from the cytosol), a difference in the pH between the two sides of the plasma and





Fig. 4.3. Carrier-mediated passive and secondary active transport. (Figure of Á. Gallé)

Fig. 4.4. Membrane transport system in plant cells (Figure of Á. Gallé)

Summary

1. Transport in plants occurs on three levels: on cellular level, tissue level (short distance transport) and whole plant (long distance transport).

2. Chemical potential of the water represents the free energy associated with water, which determines the direction of spontaneous water flow: it will move from high to low chemical potential.

3. The major factors influencing the water potential in plants are concentration, pressure and gravity, thus the water potential of solutions may be dissected into individual components. 4. Water only moves in response to water potential difference. Therefore, for water to move through the soil-plant-atmosphere system (transpiration), the Ψ_w should be as follows:

 $\Psi_{soil} > \! \Psi_{root} \! > \! \Psi_{stem} > \! \Psi_{leaf} \! > \! \Psi_{atmosphere}$

5. There are two main ways of water movement in tissues: apoplastic or symplastic transport. Water can flow through the cell walls (apoplastic transport or movement) or within cells (a symplastic route where water flows from cell to cell via plasmodesmata) or a combination of the two.

6. There are three main forces behind the water and solutes movement in the xylem: transpiration is a major component forcing xylem transport during the day; in the dark, when transpiration is low another gradient in the hydrostatic pressure (often called root pressure) forces water and solutes transportation in the xylem; water has adhesive and cohesive properties, which creates a tension (pulling) on the water in the xylem.

7. Solutes can cross membranes by simple diffusion, facilitated diffusion through channels, facilitated diffusion mediated by carrier proteins, primary active transport by pumps and *secondary active transport or* carrier-mediated cotransport (symport or antiport).
8. The gradient of electrochemical potential for H⁺ referred to as **proton motive force**

(**PMF**).

9. The proton motive force is the primary energy currency in plants: proton gradients produced by conversion of energy from high-energy chemical bonds and by the photosynthetic and respiratory electron transport chains.

Review questions

1. Why is water potential important in plant transportation?

- 2. What are the major factors influencing water potential?
- 3. Which are the main ways of water movement in tissues?

4. How can a solute cross the membrane in the direction of the electrochemical potential gradient?

- 5. What is the difference between primary and secondary active transport?
- 6. What is the pH in the cell wall, cytoplasm and vacuole?

Discussion question

1. How can stomatal closure influence the transportation in the xylem?

2. How does the pH difference between the cytoplasm and cell wall influence the membrane transport?

Additional reading

- 1. Ördög V. Molnar Z. (2011) Plant Physiology https://www.tankonyvtar.hu/hu/tartalom/tamop425/0010_1A_Book_angol_01_noveny elettan/ch03s04.html
- 2. Taiz, L. Zeiger, E. Møller, I.M., Murphy, A. (2014) Plant Physiology and Development, Sixth Edition Sinauer Associates Publication
- 3. Yeo, A. Heath, H. Sussex, W. (2007) Plant Solute Transport. Blackwell Publishing

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1. Ördög V. Molnar Z. (2011) Plant Physiology https://www.tankonyvtar.hu/hu/tartalom/tamop425/0010_1A_Book_angol_01_noveny elettan/ch03s04.html

Chapter 5. Photosynthesis, the source of life on the Planet Earth

The photoautotrophic green plants are able to utilize light energy ("photo") and to use simple, inorganic materials to produce complex organic compounds ("synthesis"). In a word, they are able to perform photosynthesis, unlike carbon-based heterotrophic organisms, which are unable to perform any of the above processes. In addition to the production of organic matter by light energy utilization, the other importance of photosynthesis is the formation of the oxygen content of the atmosphere (O_2) by which photosynthetic organisms (photosynthetic bacteria, algae and green plants) created the condition for the appearance and survival of aerobic life forms. 2.7 billion years ago, the first cyanobacteria were able to carry out oxygen-producing photosynthesis, creating the oxygen-rich atmosphere (the earliest evidence is from 2.4 billion years ago). The current volume of O_2 being present in the atmosphere is fully originated from photosynthetic activities. In addition to the fact that photosynthesis was the key to creating the oxygen-rich atmosphere, it is essential to maintain it and to help to survive for the aerobic organisms. The photosynthesis of green plants also influences the level of carbon dioxide (CO₂) in the atmosphere, as seasonal fluctuation in the CO₂ concentration of the atmosphere can be observed in parallel with the growth and decline of the vegetation. So it is easy to see that photosynthesis is a key process for our Earth's life, therefore to understand the ongoing processes is very important from both theoretical and practical considerations.

Learning objectives

- > Knowledge:
- The students know the concept of photosynthesis
- The students know to name the organisms capable of photosynthesis
- The students know the general equation of photosynthesis and the special equation of oxygen producing photosynthesis
- The students know the structure of the photosynthetic apparatus in plants, including chloroplast, pigments and photochemical systems
- The students know and understand the mechanism of electron transfer and energy transfer
- The students can explain the structure and function of the photosynthetic electron transport
- The students know and understand the Calvin-cycle
- The students are able to make difference between C3, C4 and CAM-type plants
- > Abilities:
- The students can use the obtained knowledge to recognise how environmental parameters may affect photosynthesis
- > Attitude:
- The students are open to do photosynthetic experiments to better understand plants
- Autonomy/responsibility
- The students can autonomously explain the importance of photosynthesis for life on Earth
- The students feel responsibility to provide the best possible light conditions for their plants

5.1. Utilization of light energy, photosynthetic organisms, general equation of photosynthesis

The amount of energy that reaches the atmosphere from the sun is 56 x 10^{23} J/year, from which approximately half is immediately absorbed in the atmosphere, so only the other half of it (28 x 10^{23} J/year) reaches the surface of the Earth. The energy of the wavelength that can be utilized in photosynthesis is also only approximately half of the amount that reaches the surface, about 15×10^{23} J/year. The total amount of organic matter produced by photosynthesis is 2 x 10^{11} tons/year with an energy content of 3 x 10^{21} J/year. This means that photosynthetic organisms utilize only 0.2% of the light energy that reaches the surface (1).

The ability to photosynthesise first appeared among the prokaryotes about 3.4 billion years ago. These prokaryotes (green and purple bacteria) carried out non-oxygen producing photosynthesis. One of the representatives is *Halobacterium halobium*, which absorbs photons (2) with the help of bacteriorhodopsin pigment, which is very similar to the rhodopsin found in the retina of vertebrates. The first, oxygen-producing, photosynthetic prokaryotes were cyanobacteria, which appeared 2.7 billion years ago. As an example, the unicellular *Prochloron* species can be mentioned, which are extracellular symbionts of tunicates in coral reefs of tropical seas (3). The first representatives belonged to the phylum of red and brown algae and appeared 1.2 billion years ago. Subsequently, 750 million years ago, green algae appeared, from which the first terrestrial and aquatic plants were developed approximately 470 million years ago.

We have seen that photosynthesis can take place in a variety of different ways, so the specific reactions may differ; however, a general equation could show the sequence of events occurring during photosynthesis. It is a "simple" redox reaction, in which an electron (e⁻) transfer occurs from a donor (D) to an acceptor (A), and the energy for the electron transfer is provided by the light (Reaction 1).

$$H_2D + A \xrightarrow{light} H_2A + D$$

Reaction 1: The general equation of photosynthesis

Bacteria that carry out non-oxygen producing photosynthesis can utilize hydrogen sulphide, hydrogen or various organic materials as electron donors. The vast majority of oxygen producing, photosynthetic organisms use water as an electron donor to reduce the final electron acceptor carbon dioxide. Water releases an electron while it is oxidized and an O_2 is released. It can be seen from the equation that O_2 is formed in the same amount of absorbed CO_2 (reaction 2).

Reaction 2: Reaction for oxygen producing photosynthesis

Oxygen production can also be detected when isolated chloroplasts are examined under illumination in the presence of artificial electron acceptors (Hill reaction, 4). This experiment (Hill reaction) has shown that photosynthetic O_2 production is independent of the presence (reduction) of CO_2 , i.e. two phases of photosynthesis can be separated. NADPH was already known as an electron donor, so it was obvious that in the "light phase" (without CO_2 reduction) the final acceptor of the electron transport chain was NADP⁺, so one final product is NADPH. The reduction of CO_2 occurs in the "dark phase".

5.2. Structure of the photosynthetic apparatus: chloroplasts, pigments, light absorption and excitation

Chloroplast is the cell organelle responsible for photosynthesis of higher plants. The mesophyll cells of the leaves contain 20 to 60 lens-shaped, double-membrane-bounded, prokaryotic chloroplasts. Its filled up with the stroma, which contains the CO_2 reducing enzymes, circular DNA, 70S ribosomes, and starch granules. Inside the chloroplasts, embedded in the stroma, the thylakoid membrane system is found, the stacked part of the thylakoid is called as grana-, while the freely floating parts are called the stroma thylakoids. Inside the thylakoids, a contiguous cavity system, the lumen develops. In the thylakoid membrane proteins associated with photon absorbing pigments are found, which together form the so called pigment-protein complexes.

According to the law of photochemistry, only that light can cause photochemical reaction that is absorbed by the given system, therefore a complex pigment system for light absorption is needed (Fig. 5.1.). Pigments found in all photosynthetic organisms are the chlorophylls and the carotenoids. Typical pigments of red- and cyanobacteria include phycobilins, while the non-oxygen generating, photosynthetic bacteria contain bacteriochlorophylls.

Chlorophylls can be easily extracted from chloroplasts of higher plants with acetone. Currently there are 10 types of known chlorophyll forms with different chemical structures and absorption spectra (5). The chlorophyll's structure is based on the porphyrin frame, which consists of 4 pyrrole rings and a cyclopentatonic ring, with magnesium (Mg^{2+}) ion in the middle. This ring system is equipped with conjugated double bonds, which their excitability is based on. In the chlorophyll *a* (chl *a*) molecule there is a methyl (-CH₃) group linked to the ring B while in the case of chlorophyll *b* (chl *b*) there is a formyl group (-CHO) linked to the ring B, these are the two most common chlorophyll forms. The chlorophyll porphyrin backbone is fixed to the thylakoid by the C20 phytol sidechain. The chlorophyll molecules show absorption maxima in the blue and red wavelength range, while most of the green photons are reflected. Chlorophyll *a* has two distinct forms, the P₆₈₀ with a maximum absorption at 680 nm, and the P₇₀₀ with maximum absorption at 700 nm, which catalyse the photochemical reactions. The other chlorophyll *a* and *b*, as well as carotenoids, play role only in the absorption of light and the transfer of excitation energy toward the reaction centres.

The accompanying pigments of chlorophylls are the carotenoids, they have a dual role: they pass the excitation energy towards chlorophylls and protect against light damage. They are polyisoprene molecules with 40 carbon atoms, conjugated double bonds, and α and β -ionon rings at the ends of the molecule They are yellow or red and absorbs photons in the blue and green range. They can be divided into two groups, carotenes (e.g. β -carotene) and xanthophylls containing oxygen (e.g. zeaxanthin) (6).

Following the treatment of the thylakoid membranes with detergents, two types of pigment protein complexes can be separated by gel electrophoresis. Protein complexes containing only chlorophyll a form the reaction centres and the surrounding internal antenna systems. These proteins are encoded in chloroplast and can be separated into core complex I and II (CCI and CCII). Protein complexes containing both chl a and b function as a light-harvesting antennas, and in this case the protein part is

nuclear-coded and synthesized in the cytoplasm. These can be divided for light harvesting complex I and II (LHC I and LHCII). These chlorophyll protein complexes can be arranged to photochemical system 1 (photosystem I, PSI) and photochemical system 2 (photosystem II, PSII).

The PSII can be separated to four functional parts: reaction centre, regulating cap, proximal antenna, distal antenna. The reaction centre is formed by the D1 and D2 heterodimeric proteins having 5-5 transmembrane segments and 353 amino acids. In the reaction centre, there is a P_{680} dimer, further chlorophyll *a*-s and two pheophytin-*a* (primary acceptors). The D1 protein contains a tyrosine amino acid side chain (Y₂), which is the primary electron donor to P_{680}^{+} . Further components of the D1 and D2 proteins are plastoquinone A and B (PQA and PQB). The reaction centre also contains a photoprotective cytochrome b559 and water-oxidizing Mn atoms. The regulating cap is connected to the reaction centre of the PSII on the lumen side. It consists of six (two 33, two 23, two 17 kDa) proteins, and probably provides Ca²⁺ and Cl⁻ ions required for water oxidizing. The proximal antenna consists of two chlorophyll protein complexes (CP43 and CP47) and their role is to transmit the energy of light to the P₆₈₀. The distal antenna system is located further from the reaction centre, and it is build up from LHCII and an additional chlorophyll protein complexes. In LHCII, the ratio of cl *a* and cl *b* is 1: 1, in addition it contains further xanthophylls, while in the chlorophyll protein complexes the ratio of cl *a* and cl *b* is 2: 1 (7).

The reaction centre of the PSI consists of two proteins (the 83 kDa PsaA and the 82 kDa PsaB), containing P_{700} with further 40-50 chlorophyll molecules, and 1-2 β -carotene. In addition to these pigments, in the PSI reaction centre there can be found the A₀ chlorophyll *a* monomer, the A1 (vitamin K or phylloquinone) and ferredoxins (FeSx, FeSA, FeSB). PSI contains a ferredoxin (Fd) binding and a plastocyanin (PC) binding region that interact electrostatically with molecules (8).

Figure 5.1. (A) When a molecule absorbs a photon (photon absorption), it absorbs its energy and gets to a higher energy level, i.e. it is excited. (B) In an excited state, a delocalized electron is boosted to a shell farther from its atomic nucleus. (Figure of Z. Kolbert)

In the case of P_{680} and P_{700} chlorophyll *a*-s, the electron transferred to the relaxation electron shell is passed to a nearby acceptor which is reduced while the cl *a* itself is oxidized. From a nearby donor, the chlorophyll *a* will be reduced by another electron. Thus, a light energy-mediated electron transfer takes place via the P_{680} and P_{700} chlorophylls, which is called photosensitized electron transfer. Only two forms of chlorophyll molecules (P_{680} and P_{700}) can be involved in the charge separation reactions, as they exist in specific molecular environments in the reaction centres that allow direct contact with the electron donor and acceptor.

What is the role of the other chl *a*, chl *b* and carotenoid pigments in light absorption? These pigments, after absorbing the energy of the photon, return from the excited energy state to the basal state, while

the excess energy is transferred to an adjacent chlorophyll (energy transfer), which gets thereby excited. When it occurs between chemically identical molecules (from chl *b* to chl *b*), the energy transfer is called energy migration. The energetic criterion of the transfer is that the excitation spectra of the donor and the emission spectra of the acceptor should be overlapping. The greater the overlap between the spectra, the more efficient the energy transfer is. Furthermore, energy transfer is more effective when the pigments are close to each other. Energy transfer takes place between pigment molecules (chl *a* and chl *b*) within the same system only in the case when the excitation wavelength characteristic of the first pigment is overlapping with the emission of the acceptor molecule. The energy transfer takes place from the direction of the pigment that absorbs at the shorter wavelength towards the pigment absorbing at the longer wavelength. In the case of chl *a* and chl *b*, the overlap of the absorption-emission spectra and the localization of the pigments in high concentrations in the reaction centres is given, therefore the energetic and spatial conditions of the energy transfer are fulfilled.

In light-harvesting antenna systems, the order of the energy transfer is as follows: carotenoids, chl *b*-s and chl *a*-s, and then the final step is the reaction centre pigment itself (P_{680} or P_{700}), which is having an absorption maximum at longer wavelength than the surrounding pigments, and therefore receives energy from them, i.e. works as an energy trap. The P_{680} and P_{700} themselves are able to absorb photon, but because of their small number (only 1-2% of the total chl *a* content), the amount of photon absorbed by them is only 1-2% of the photons absorbed by the entire apparatus. The system is made effective by the energy transfer in antennas by repeatedly exciting the P_{680} and P_{700} , thus increasing the frequency of photochemical reactions (Figure 5.2; 9).

Antenna complex

Figure 5.2. The concept of energy transfer. The huge amount of pigments in the antenna complexes collect light energy and transmits it towards the reaction centre, where an electron is transferred to the acceptor during the photochemical reaction. Subsequently, an electron donor recirculates the missing electron of the reaction centre chlorophyll. Transmission of light energy in the antenna system is a purely physical phenomenon, chemical transformations are not involved. (Figure of Z. Kolbert)

5.3. Operation of photosynthetic electron transport chain

Considering the two photochemical systems and the chloroplast, cytochrome, quinone and ferredoxintype compounds, Hill and Bendall (10) formed the Z-scheme of the photosynthetic electron transport chain. The scheme shows the flow of electrons from water to NADP⁺ and the changes in the redox potential of each component. The redox potential indicates the ability of a molecule to electronize, i.e. to reduce. The redox potential is marked as "E", while it's unit is volt (V). The redox potential of hydrogen by convention is 0. In case of higher electron capture capability from the hydrogen, E has a positive sign (it is capable to oxidize other molecules) and, in the case of a smaller capability, has a negative sign, at which point the molecule "prefers to deliver electron" (to reduce other molecules).

The ground state P_{680} chl *a* has a redox potential of +1.2 V, whereby the electron accepting capability is high. As a result of excitation following photon absorption, the redox potential is reduced (-0.6 V), whereby the ability of the electron to release is increased towards the primary acceptor. The redox potentials of other members of the electron transport chain are higher values, i.e. they tend to pick up an electron from the previous components. The P_{700} chl has a resting redox potential of +0.45 V, allowing it to capture electrons flowing from PSII. As a result of excitation, the E value drops to -1.4 V, so electron release occurs to the primary acceptor. The redox potentials of other members of the electron transport chain take on increasing values, so the transfer of electrons from molecule to molecule and then to the final acceptor (NADP⁺) can take place. Figure 5.3. shows the Z-scheme of the photosynthetic electron transport chain.

Figure 5.3. Z-scheme of the oxygen-evolving photosynthetic electron transport chain. Explanation in the text. Abbreviations: Pheo Pheophytin; PQA plastoquinone A, PQB plastoquinone B, FeSR Rieske iron sulphur protein, Cyt f cytochrome f, Cyt b cytochrome b, PC plastocyanin, Fd ferredoxin, FNR ferredoxin NADP⁺ oxidoreductase. (Figure of Z. Kolbert)

The primary e^- acceptor in the reaction centre of PSII is the pheophytin, which is reduced by e^- uptake where the P₆₈₀ is oxidized. The e^- goes from pheophytin to PQ_A, and then to PQ_B. The latter molecule takes up two electrons and two protons (H⁺) from the stroma, and is reduced to QBH₂ and then detaches off from the reaction centre of PSII to become the first mobile component (PQH₂). Quinones are conjugated cyclic dions (containing two ketone groups). Their small size and apolar nature allow their free diffusion in the lipid phase of the thylakoid.

The reduction of P_{680}^+ , which was oxidized during the charge separation reaction, is carried out by the electrons released from the water. On the lumen side of PSII, an Mn-containing water oxidizing complex (OEC, oxygen evolving complex or M-enzyme) is localized. According to the oxidation states

of the Mn ion, the enzyme can have four states (S0, S1, S2, S3, S4, which is immediately converted to S0), which collect positive charges for the water breakdown. In a cycle, 4 e^{-} passes from two water molecules to Y_z (tyrosine amino acid in the D1 protein) and then to oxidized P₆₈₀⁺, and requires 4 photons to be absorbed in the PSII reaction centre. Meanwhile, an O₂ molecule is released and 4 H⁺ is released into the thylakoid lumen (11).

The mobile PQH₂ binds to the lumen-side of the cytochrome b6/f complex and then puts its two protons into the lumen. One of the two e⁻ delivered to the complex is transferred to the cytochrome f via the iron-sulphur protein (Fe-SR) and then to the mobile PC; from the PC, the excited, e⁻-deficient P_{700} (+0.45 V) takes over the electron.

The other e⁻ goes into the so-called Q-cycle, firstly to the cytochrome b6 (with low redox potential), then to the high redox potential heme group and finally back to the PQ. With an e⁻ uptake, a semiquinone (Q⁻⁻) is formed, and after binding of a second PQH₂ to the cytochrome b6/f complex, a new PQH₂ is formed by the addition of another e⁻ and two H⁺. The essence of the Q cycle is therefore to increase H+ translocation from the stroma to the lumen, which is important in ATP synthesis (see below).

Inside the PSI the excited P700 passes on the electron to the primary acceptor A0 localized in the reaction centre, which transmits it to A1. The bound ferredoxins (Fd, proteins containing an iron-sulphur prosthetic group) take over the electron (FeSx, FeSA and FeSB, respectively), and finally passes to the mobile ferredoxin, which, in the case of a linear transport chain, transmits the electrons to ferredoxin-NADP⁺ oxidoreductase (FNR). FNR is a 33 kDa nuclear-coded enzyme that is localized to the stromal side of the thylakoid membrane. FAD binds to it as a prosthetic group, and there is also an NADP and an Fd binding site on the enzyme. The enzyme supplies two electrons to NADP+, to do so the enzyme oxidizes two Fd in two successive steps. When the semiquinone-FNR complex is formed between the first molecule of oxidized Fd and the enzyme, the oxidized Fd is released, and replaced by a reduced one. The two e⁻ uptake is followed by two H+ binding, resulting in a reduced NADPH, which is one of the stable end products of the linear electron transport chain.

If the electron returns from the mobile Fd to the PQ, the transport chain will be cyclical. In this case, H^+ translocation from the stroma to the lumen is enhanced, favouring the ATP formation. While in the case of linear electron transport, water is oxidized and both photochemical systems are active, in the case of cyclic electron transport only PSI is excited and "drives the electrons around the reaction centre". Then O_2 and NADPH are not produced, but ATP does. With two types of electron transport chain, the system can set the ratio between of the NADP and ATP production.

In addition to oxygen evolution and reduced coenzyme production, ATP is the third product of the photosynthetic light reactions. The prerequisite for ATP synthesis is the formation of the H⁺ concentration difference (gradient) between the two sides of the thylakoid membrane. Inside the thylakoid lumen H⁺ concentration increases during the light reactions, as H⁺-s come from water decomposition and H⁺-s are transported by the PQH₂ from the stroma to the lumen. The difference in H⁺ concentration is used up by the ATP synthase enzyme located in the membrane, which directs H+ movement from the lumen to the stroma (12). The function of the CF₀-CF₁ type ATP synthase is to convert the electrochemical energy of the proton gradient between two sides of the thylakoid membrane into ATP.

5.4. Synthesis and export of stable products

The next step in photosynthesis is the carbon fixation, where CO_2 is converted to stable, carbon based organic molecules. The energy and the reducing power is utilized by the formerly synthetized stable products (ATP and NADPH). The synthetized stable products are used in long-term energy storage during the biosynthesis of carbohydrates.

Carbon dioxide is captured in a cycle of reactions known as the Calvin cycle or the Calvin-Benson cycle after its discoverers. It is also known as just the C_3 cycle. The cycle takes place in the stroma, where all the necessary enzymes are found. CO_2 diffuses into the stroma of chloroplasts and is combined with a five-carbon sugar, ribulose 1,5-biphosphate (RuBP) to form an intermediate product, which is immediately degraded to two molecules of the 3-carbon compound 3-phosphoglyceric acid (3PGA). The enzyme that catalyses this reaction is referred to as Rubisco (Ribulose-Bisphosphate Carboxylase/Oxygenase), a large, slow reacting molecule that may be the most abundant protein on the Earth (it gives half of the proteins in a leaf itself). The fact that this 3-carbon molecule is the first stable product of photosynthesis leads to the practice of calling this cycle as the C₃ cycle. This first step is the carbon fixation. During the next step (reduction) energy and reducing power is used from ATP and NADPH to remove a phosphate group from 3PGA and reduce the resulting diphosphoglycerate (DPGA) to produce the 3-carbon sugar glyceraladehyde-3-phosphate (G3P). Some of this G3P is used to regenerate the RuBP to continue the cycle (regeneration), but some is available for molecular synthesis and is used to make glucose, fructose, sucrose, starch and other carbohydrates (Figure 5.4.)

glucose, fructose, sucrose, others

Figure 5.4. The Calvin cycle (Figure of Z. Kolbert)

In C3 plants the photosynthesis, carbon fixation and Calvin cycle all occur in a single chloroplast. In C4 plants the photosynthesis takes place in a chloroplast in separated cells, carbon fixation occurs in the mesophyll cells, where the first stable product is a 4-carbon organic acid, which is then transported to a bundle sheet cell, where the Calvin cycle occurs. This protects the Calvin cycle from the effects of photorespiration. In this case the carbon fixation and the Calvin cycle is separated by space. In CAM plants the photosynthesis and initial carbon fixation occur at night and a 4-carbon acid is stored in the cell's vacuole. During the day, the Calvin cycle operates in the same chloroplasts. In this case the carbon fixation and the Calvin cycle are the carbon fixation and the Calvin cycle is separated by time.

Summary

- 1. During photosynthesis, plants utilize light energy to synthesize complex organic molecules (glucose) from simple inorganic material (CO₂), while producing oxygen and also affecting the level of CO₂ in the atmosphere.
- 2. There are bacteria, algae, and higher plants among photosynthetic organisms. Bacteria include non-oxygen generating photosynthesising species and oxygen producing cyanobacteria. During photosynthesis of higher plants oxygen is always released.
- 3. During oxygen-producing photosynthesis, water (electron donor) is oxidized and CO_2 is reduced as an electron acceptor to produce carbohydrates.
- 4. The Hill reaction proved that photosynthetic O₂ production and CO₂ reduction are independent processes, that is, photosynthesis can be separated into "light" and "dark reactions".
- 5. For higher plants, photosynthesis occurs in the thylakoid membrane system (light reactions) and stroma (dark reactions) of chloroplasts. Chlorophyll and carotenoid pigments are responsible for photon absorption.
- 6. The pigments are found in pigment protein complexes in thylakoids, which forms the photosystem 1 and 2 (PSI and PSII).
- 7. Inside the reaction centre of the photosystems two specific chlorophyll molecules, P_{680} and P_{700} can be found, which provide electron transport with charge separation (catalyse the photochemical reaction). The other forms of chlorophyll and carotenoids are responsible for the transmission of the excitation energy to the P_{680} and P_{700} .
- 8. The Z-scheme of the photosynthetic electron transport chain depicts the redox potential changes of the components. As a result of excitation, the large redox potential of P_{680} and P_{700} is reduced, allowing them to deliver electrons to the acceptor molecules.
- 9. Components of the photosynthetic electron transport chain and direction of electron transfer: water $\rightarrow Y_Z \rightarrow P_{680} \rightarrow$ pheophytin $\rightarrow PQ_A \rightarrow PQ_B \rightarrow$ cytochrome
 - b6/f \rightarrow plastocyanin \rightarrow P₇₀₀ \rightarrow A0 \rightarrow A1 \rightarrow FeSx \rightarrow FeSa \rightarrow FeSB \rightarrow m ferredoxin \rightarrow FNR \rightarrow NADP⁺
- 10. The electron deficiency due to excitation of the P_{680} chl *a* is recovered by the electrons from the water oxidation. The water-oxidising M-enzyme can take 4 states while removing 4 electrons and 4 protons from 2 water molecules, while an O_2 is released.
- 11. The basis of ATP formation is the difference in the H+ concentration between two sides of the thylakoid during the operation of the electron transport chain, which is finally eliminated by the ATP synthase enzyme during ATP formation.
- 12. Products of the linear electron transport chain: O₂, ATP, NADPH, cyclic electron transport chain products: ATP.
- 13. During the Calvin cycle, CO_2 is fixed with the help of the Rubisco enzyme, and all the stable products are used up, which were synthetized during the light reactions.
- 14. The final products of the photosynthesis are carbohydrates, which are capable for long-term storage.

Review questions

- 1. How does photosynthesis of green plants affect the composition of the atmosphere?
- 2. What structural properties of pigments make photon absorption and excitation possible?
- 3. What is the difference between electron transfer and energy transfer? Which molecules occur in which part of the photosynthetic apparatus?
- 4. What is the essence of the Q-cycle in the cytochrome b6/f complex?
- 5. What is the reason behind the fact that the photosynthetic electron transport chain has a linear and cyclic form?
- 6. What kind of change does the excitation following photon absorption cause in the redox potential of the P680 and P700?
- 7. What is main role of the RuBiSCO? Why a plant needs so much of them?

Issues to be discussed

1. What are the practical significance and application fields of knowledge about photosynthesis of plants?

Suggested reading

- 1. Taiz L, Zeiger E (szerk.). Plant Physiology 5th edition. Sinauer Associates Inc., USA, 2010.
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- 3. Robert E. Blankenship: Molecular Mechanisms of Photosynthesis

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Chapter 6. Plant hormones

In this chapter, those endogenous plant growth regulators (hormones) will be introduced and generally discussed, which coordinate plant growth, development and environmental adaptation. Their overall characteristics discriminating them from the animal hormones, their chemical nature, structure, perception, and function will be overviewed.

Learning goals:

- > Knowledge:
- The students know the term "plant hormone"
- The students know to name the plant hormones
- The students are able to compare plant and animal hormones and they understand the main differences
- The students understand the processes regulating plant hormone levels and actions
- The students have a general view about plant hormone perception and signal transduction and their specificities
- > Abilities:
- The students can use the obtained knowledge to recognise hormonemetabolism/perception/transport-related problems during plant growth and development
- > Attitude:
- The students are open to study the role of plant hormones in plant development and adaptation
- Autonomy/responsibility
- The students can autonomously explain the importance of plant hormones in plant life
- The students can independently argue that plant hormones can indeed be classified as hormones despite their differences from the animal ones

6.1. Definition and general characteristics of plant hormones

Plant hormones are organic compounds synthesized by plant cells exclusively having signal transduction function to coordinate plant growth, development and environmental responses (1). In general, they are effective in small concentrations (10⁻⁹ M to 10⁻⁶ M) and are perceived by specific receptor molecules either at the cell surface or inside the cells.

Based on these criteria, currently we consider as plant hormones the following nine organic molecules (Fig. 6.1): auxins (AUX), cytokinins (CK); gibberellins (GA); abscisic acid (ABA); ethylene (ET); brassinosteroids (BR); salicylic acid (SA); jasmonic acid (JA); strigolactone (SL). The list could be extended by several hundred small peptide molecules that have signal transduction functions in plants. These are collectively termed as "plant peptide hormones" (Fig. 6.1). Their function is rather diverse and only a few of them have been characterized until know. Their primary role is cell-to-cell communication.

Figure 6.1. The structure of plant hormones. (Figure of A. Fehér)

There are several other molecules in plants that are although important for plant growth, development, and adaptation, their signal transduction function is only secondary and have primary role in plant metabolism. Such kind of growth regulators are the polyamines, the hydrogen-peroxide, the nitric oxide, sugars, certain amino acids etc. The signal transduction function of these molecules does not depend on specific receptors and often act only at large concentrations (10⁻⁶ M to 10⁻³ M).

Plant and animal hormones are rather different in many aspects.

The chemical nature of animal hormones is rather variable. Among others, modified amino acids (e.g. norepinephrine), peptides (e.g. oxytocin), steroids (e.g. progesterone), and large extracellularly sensed proteins (e.g. growth factors) can have hormone function in animals. These animal hormones are produced in specific organs, the glands, and are delivered to distant target organs, tissues, or cells via the blood stream. Their production and function are centrally coordinated by the nervous system.

In contrast, the nine classical plant hormones are all small organic molecules, which directly or indirectly can enter into plant cells (1). They can be synthesised practically in any plant cell, although certain organs, tissues, cells can produce more than the others. Normally they act over a short distance, although they can also be delivered to distant organs in the xylem or phloem. Plant hormones have no specific target cells; they influence all cell type they interact. Moreover, they have no exclusive tasks, they influence all aspects of plant life with characteristic major roles in a subset of plant processes. They are not centrally regulated but form complex hormonal networks modifying each other's functions. Instead of a single hormone, their combination is that gives specificity for their action.

Figure 6.2. Biosynthetic pathways of plant hormones in relation to general plant metabolism. (Figure of A. Fehér)

6.2. The processes influencing plant hormone levels

Plant hormones are biosynthesized via rather general metabolic pathways serving other primary roles (1) (Fig. 6.2). The precursors of auxin, ethylene, and salicylic acid are amino acids such as tryptophan, methionine, and phenylalanine, respectively. Plant peptide hormones are produced by the cleavage of protein precursors. Cytokinins are adenine derivatives, while jasmonic acid is synthesized from α -linoleic acid, a fatty acid present in cell membranes. All the further plant hormones are the by-products of secondary metabolism producing terpenoid (isoprenoid) molecules. Abscisic acid and strigolactone are formed by the oxidative cleavage of 40-carbon-atom-long carotenoid precursors. Gibberellic acid derives from the general precursor of diterpenoid biosynthesis, geranylgeranyl pyrophosphate. Brassinosteroids are biosynthesized from campesterol, an abundant constituent of plant cell membranes.

Except ethylene, salicylic acid and abscisic acid, all plant hormones can exist in plants in various forms. This structural diversity is mainly due to conjugation (glycosylation, amino acid conjugation) or methylation (2). The modified forms may have different physiological activities than the basic hormone or can serve as inactive storage forms from which the free unmodified active hormone can be quickly released if needed. This reversible feature allows the fine tuning of hormone levels without the requirement for new synthesis.

The hormone levels, however, are mainly defined by the equilibrium between biosynthesis and degradation (1). The rate of degradation is related to hormone action: fast, few minutes' hormone actions are associated with rapid degradation (e.g. wound response), while long-term developmental processes (e.g. germination, seed filling etc.) require persistent hormone action and therefore long hormone half-lives. Hormones are degraded by specific enzymes (especially oxidases, hydroxylases). For ethylene, a gaseous plant hormone, no degradation pathway exists, since its level is mainly determined by synthesis and diffusion into the air. Strigolactone is also a special case since its receptor itself degrades the hormone and thus one hormone molecule can activate one receptor and only once.

Like for animal hormones, the sites of biosynthesis, storage, and function of plant hormones might also be different. Therefore, plant hormones might also need to be transported within the plant (3). Plant hormone transport might include intracellular, cell-to-cell, and long-distance transport mechanisms. Plant hormones that are week acids, such as auxin and abscisic acid, can enter the cells through the plasmamembrane via simple diffusion. However, in most of the cases specific transporter molecules aid and regulate plant hormone movement within and between cells. These are mainly permeases, carriers, channel proteins, or ATP-dependent pumps. Plant hormones can enter and leave the vasculature, xylem and/or phloem, via cell-to-cell transport and can be delivered by the flow of water and nutrients over long distances. The auxin, however, can move a long distance via a directed cell to cell transport mechanism. The auxin gradients established in this way are very important for plant morphogenesis and development. This mechanism is based on the polar localisation of specific auxin afflux carrier proteins in the cell membrane, allowing the auxin to leave the cell only in one direction.

6.3. Sensing and signalling of plant hormones

It is a general characteristic of hormones, animal and plant alike, that their action is dependent on their concentration. The strength of certain hormone actions is proportional with the hormone concentration (e.g. plant cell elongation in response to auxin), while in other cases hormones act like "on/off" switches: above a threshold concentration they evoke a full response (e.g. dormancy breaking by gibberellin). In other words, hormone responses can be quantitative (e.g. growth) or qualitative (e.g. initiation of flowering). Moreover, they can be reversible (e.g. opening of stomata) or irreversible (e.g. lateral root formation).

Beside hormone concentration, the response is dependent on the sensitivity against the specific molecule. This sensitivity is different among plant species, developmental states, organ, tissue, and cell types etc. Hormone sensitivity relies on the efficiency of hormone-triggered signal transduction fundamentally determined by hormone-receptor interactions. Therefore, in addition to hormone concentration, hormone sensitivity is influenced by the number of receptor molecules (per cell, tissue, or organ) in relation to the hormone concentration, their availability for interaction (compartmentalisation, occupation of binding sites, etc.), affinity of the receptor towards the hormone molecule (receptor conformation, post-translational modifications et.), the presence of inhibitor molecules etc.

Plants have various types of hormone receptors (4,5) (Fig. 6.3). They can be membrane-bound (BR, CK, ET, peptides) or soluble (AUX, GA, JA. SA, SL, ABA). Among the transmembrane receptors, BRs and the peptides (and maybe CKs) have cell surface receptors inserted into the plasmamembrane, but ET and CKs have their receptors sitting in the membrane of the endoplasmic reticulum (ER). Soluble plant hormone receptors are either cytosolic (ABA, GA, SL, SA) or nuclear (JA, AUX) or can move between these two compartments (GA, SL, SA).

The signal transduction triggered by activated plant hormone receptors either involves protein phosphorylation/dephosphorylation or proteosomal protein degradation except for salicylic acid that is based on conformational changes in its receptors having direct transcriptional co-regulator activity (Fig. 6.3).

Figure 6.3. Plant hormone signalling pathways. (Figure of A. Fehér)

The first group consists ABA, CK, ET, BR, and peptides. BR and peptides are sensed by transmembrane serine/threonine receptor kinases having an extracellular ligand-binding, a transmembrane, and a cytosolic serine/threonine kinase domain (unlike animal receptor kinases that have a tyrosine kinase domain at the cytosolic end). In case of ligand binding, they can phosphorylate various intracellular substrates mediating the signal transduction. These substrates are often cytoplasmic protein kinases or kinase cascades that can process and amplify the signal. The cytokinins and ethylene are perceived by histidine receptor kinases, an enzyme class that is not represented in animal organisms (but are present in yeast). Despite the similarity of the receptor, the signal transduction pathways of CKs and ET are different.

The signal transduction mechanism of CKs resembles the two-component system of prokaryotic organisms. This is based on phosphotransfer reactions between dedicated histidine and aspartate amino acid residues of three types of proteins. In response to ligand binding, the histidine kinase autophosphorylates on a defined histidine residue. The phosphor is then transferred to and aspartate residue at the C-terminal end of the same receptor molecule. From here, it is transferred again to a histidine amino acid on a small transfer protein that enters into the nucleus and transfers the phosphor to an aspartate of a so-called response regulator. The response regulator might have a transcription activator domain to initiate gene expression binding to specific promoters or might not have it serving as a competitive negative regulator of transcription.

The ethylene receptors are also histidine kinases. Interestingly they are active in the absence of ethylene and get inactivated via ligand binding (negative regulation). In the absence of ET, they activate a downstream intracellular kinase called CTR1. CTR1 phosphorylates a membrane-bound protein EIN2 that is inactivated in this way and there is no further signal transduction, the master transcription factors of ethylene signalling (EIN3/EIL1) get degraded. In the presence of ethylene that binds to the receptor, the histidine kinase goes through a conformational change and the CTR1 kinase cannot be activated. The EIN2 protein is therefore not phosphorylated but cleaved by a protease. The C-terminal

part of EIN2 is released from the cell membrane, enters the nucleus and prevents the degradation of EIN3/EIL1 to allow transcription from ethylene-regulated promoters.

The receptor of ABA is a small cytosolic protein that if binds the hormone changes conformation and efficiently inhibits a PP2C-type protein phosphatase. If the phosphatase is not active, it cannot dephosphorylate the SnRK kinases, which auto-phosphorylate themselves. The auto-phosphorylated kinases get high activity and phosphorylate their substrates, which are the transcription factors implicated in ABA signalling. The phosphorylated transcription factors are saved from degradation and thus can perform their gene regulatory function.

The signal transduction of AUX, JA, SL, and GA hormones proceeds via proteosomal degradation of inhibitory proteins. Proteins are targeted for proteosomal degradation if covalently conjugated to the small protein molecule(s), ubiquitin. The ubiquitination of proteins requires three activities mediated by different enzymes/enzyme complexes: activation of ubiquitin (E1), presentation for the activated ubiquitin to the ubiquitin ligase complex (E2), and covalent attachment of the molecule to the target by this complex (E3). The ubiquitin ligase complex has a specific protein, the F-box protein, that is responsible for substrate recognition and binding. The AUX and JA hormone receptors are F-box proteins which can specifically recognise their targets only if they bind the appropriate hormone molecule: the hormone molecule actually serves as a glue between the F-box protein and its substrate. The substrates are transcriptional inhibitors that in the absence of the hormone prevent transcription regulated by specific transcription factors sitting on hormone-regulated promoters. The presence of the hormone targets these inhibitors for degradation allowing fast gene activation. SL and GA signal transduction differs only slightly that of AUX and JA. In these cases, the hormone receptor is a small protein that if binds the hormone get attached to an F-box protein allowing it to recognise its target. So, the GA and SL hormone is indirectly linked to the corresponding F-box protein.

6.4 The biological function of plant hormones

Plant hormones act in concert with each other (6). They often exert their effects regulating the levels and signal transduction of other hormones. Therefore, it is very difficult to assign the regulation of any biological process to a single plant hormone. Nevertheless, all plant hormones have certain roles in plant life that are characteristic for them and considered as their primary function (4).

Auxin is probably the best characterized plant hormone with basic roles in plant growth and development. At the cellular level, auxin is responsible for the promotion of cell elongation and, together with cytokinin, cell division. Therefore, the development of plant form (morphogenesis) is strongly affected by local auxin concentrations, which are formed besides localised auxin synthesis, conjugation and degradation by directed long term auxin transport. Auxin has its role as a morphogen from the initiation of embryogenesis specifying the apical-basal polarity of the plant throughout plant development maintaining the functioning of meristems and defining the sites of organ (leaf, lateral root) initiation and inhibiting shoot branching.

Cytokinins are also required for cell division and organ formation acting together with auxin. While both hormones promote cell division, they often have complementary accumulation patterns and antagonistic roles during morphogenesis. While exogenous auxin induces root formation in cultured callus tissues, cytokinin treatment results in shoot regeneration. Cytokinin delays senescence and enforces the metabolic sink characteristics of organs, tissues. Cytokinin produced in the root communicates towards the shoot the availability of nutrients. Strigolactones are recently discovered plant hormones with primary roles in shoot and root branching. However, more and more data accumulate that support a more general role of these compounds in the regulation of plant development and stress tolerance.

It is the best-known function of gibberellins that they promote shoot elongation. Gibberellin biosynthesis or signalling mutants are dwarfs. GAs often antagonise the action of abscisic acid breaking dormancy states, e.g. in seeds. GAs positively regulate seed germination, the initiation of flowering, and fruit development.

Brassinosteroids are also involved in the positive regulation of shoot growth; mutants with inhibited brassinosteroid production/responses are dwarfs. Moreover, these mutants exhibit a constitutive photomorphogenic phenotype: their seedlings grow in the dark as they would grow in the light. Root growth is also regulated by BR, but in a concentration-dependent way (high concentrations inhibit root growth). BRs promote xylem differentiation, flowering, pollen development and pollen tube growth, and in certain cases promote programmed cell death and senescence. BR increases abiotic stress tolerance and coordinates growth with pathogen defence responses.

Abscisic acid is primarily considered to be a stress hormone, but it is also involved in developmental regulation including the control of seed maturation and dormancy, senescence and fruit ripening. ABA activates genes that enhance abiotic stress, including drought and cold stress, tolerance. Moreover, ABA regulates stomatal closing in response to water shortage and coordinates root and shoot growth under drought conditions.

Another plant hormone that is best known about its role in stress responses is ethylene. It inhibits plant growth and activates defence genes under stress conditions. It enhances senescence and leaf abscission and has an important role in the ripening of fruits. At the cellular level ET regulates cell elongation.

Salicylic acid is also involved in abiotic stress responses, but its main role is in pathogen tolerance. It activates pathogen resistance (PR) genes and is also involved in the hypersensitive reaction of plant cells. Cells around the infection site go through programmed cell death to prevent spreading of the pathogen. Moreover, SA promotes senescence and is responsible in certain plants and tissues for heat production.

Jasmonic acid negatively regulates growth, germination, and flowering, but promotes leaf senescence, fruit ripening and the formation of storage organs. In addition to its developmental roles, it functions in the defence against herbivores.

Summary

- Plant hormones are organic compounds synthesized by plant cells. Their only role is signal transduction to coordinate plant growth, development and environmental responses. They are effective in small concentrations are perceived by specific receptor molecules.
- 2. The nine main plant hormones are: auxins (AUX), cytokinins (CK); gibberellins (GA); abscisic acid (ABA); ethylene (ET); brassinosteroids (BR); salicylic acid (SA); jasmonic acid (JA);

strigolactone (SL). Plant peptide hormones represent another class with several hundred members.

- 3. In contrast to animal hormones, plant hormones: 1) are not synthesized in specific glands; 2) have no specific target cells; 3) are not under centralised control; 4) have many functions and are less specific.
- 4. The levels of active plant hormones are determined by the rate of biosynthesis, inactivation/degradation, conjugation, and transport.
- 5. Plant hormones are biosynthesized via rather general metabolic pathways serving other primary roles: amino acid synthesis, nucleotide synthesis, lipid synthesis, terpenoid (isoprenoid) synthesis.
- 6. Plant hormone receptors are mostly intracellular (brassinosteroids and peptides have cell surface receptors, cytokinin has both intra-and extracellular ones).
- 7. The signal transduction triggered by activated plant hormone receptors either involves protein phosphorylation/dephosphorylation or proteosomal protein degradation except for salicylic acid that is based on conformational changes in its receptors having direct transcriptional co-regulator activity.
- 8. Plant hormones act in concert with each other. They often exert their effects regulating the levels and signal transduction of other hormones. They have several overlapping functions coordinating plant development with environmental adaptation.
- 9. Plant hormones are characterized by their best-known biological functions. Auxins and cytokinins are primarily responsible for the regulation of growth and morphogenesis. Brassinosteroids and gibberellins positively regulates growth and influences cell/tissue differentiation and plant development. Abscisic acid and ethylene are mainly considered as stress hormones. Salicylic acid is important for the defence against pathogens, jasmonic acid against herbivores. Strigolactones are less characterized but have important roles in shoot/root branching. Peptide hormones are involved in short-range cell-to-cell communication. In general plant hormones that have primary role in stress/defence responses promote maturation and senescence, while those positively regulating growth maintain juvenility, break dormancy and delay senescence.

Questions

- 1. What is the definition of plant hormones?
- 2. List the most important plant hormones!
- 3. What are the main general differences between plant and animal hormones?
- 4. What are the processes determining the local levels of plant hormones?
- 5. What type of biosynthetic pathways plant hormones have?
- 6. Which plant hormones are: not transported; transported from cell-to-cell over a large distance; transported in the xylem or phloem?
- 7. What the conjugation of plant hormones means and what is its significance?
- 8. What type of receptors plant hormones have?
- 9. Which are the plant hormones having primary function regulating plant morphogenesis and which are those primarily involved in stress tolerance and defence?
Questions to discuss

- 1. How the various plant hormones interact with each other?
- 2. How plant hormones are used in agri/horticulture?

Suggested reading

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Chapter 7. Light perception

In this chapter it will be discussed how plants can sense light, an important environmental parameter thoroughly affecting plant development.

Learning goals:

- > Knowledge:
- The students understand how light affects plant life and development
- The students know that which parameters of light irradiation plants can sense and discriminate
- The students can list plant photoreceptors and briefly characterize them
- > Abilities:
- The students can use the obtained knowledge to recognise how light parameters may affect plant growth and development
- > Attitude:
- The students are open to investigate the effect of light on plant development
- Autonomy/responsibility
- The students can autonomously explain the importance of light for plants beside photosynthesis

7.1. Light and plant development

Light is important for plants not only as a source of energy. Light carries various information about the environment surrounding the plants such as the time of the day, the season, population density, direction of light, degree of shadow etc. (1,2). Plants have no specific organ to sense light but have various photoreceptors in many of their tissues/cells. Plant photoreceptors are capable to sense many parameters of light including wavelength, intensity, quality, direction, periodicity etc. Photoreceptors trigger signal transduction leading to short-term physiological and gene expression responses and long-term growth and developmental changes. Photoreceptors mediating light effects regulate among others germination, photomorphogenesis of seedlings, phototropism, stomatal movement, chloroplast movement and orientation, circadian rhythm, flowering.

When light passes through the leaf, the blue and red part is absorbed for photosynthesis. Far-red light although mostly passes through the leaf also carries information and therefore plants have evolved photoreceptors sensing the important UV-A-to-blue and red-to-far-red portions of light. In addition, they can sense the deleterious UV-B spectrum to induce defence and adaptation.

7.2. Photoreceptors

7.2.1. Sensing red and far-red light by phytochromes

7.2.1.1. The structure of phytochromes

Phytochromes are present in all plants developing in light. These are large molecules, the holoprotein is app. 120 kDa in size. The functional photoreceptor is the dimer of two holoproteins (3,4). The structure of phytochromes is rather complex (Fig. 7.1.). They have a C-terminal and an N-terminal region linked with a hinge. The N-terminal region contains several functional domains: a serine-rich signal transduction domain (NTE) and three domains (designated PAS, GEF and PHY) forming the

photosensory core. This core has a bilin lyase activity and links the bilin chromophore to the GAF domain via a thioether bound. The bilin chromophore is called as phytochromobilin. The chromophore goes through a cis-trans isomerisation in response to light that cause a conformation change in the light-sensing domain as well as the whole molecule. The C-terminal region has two PAS-domains (forming together the PAS-related domain, PRD) and the histidine-kinase-related domain (HKRD). The PAS domains have role in dimerization and interaction with other proteins while the HKRD domain possesses protein serine/threonine kinase activity implicated in signal transduction.



Figure 7.1. Basic structure of the phytochromes PHYA. Legend: H, hinge; NTE, N-terminal extension; PAS, Per (period circadian protein), Arnt (Ah receptor nuclear translocator protein), and Sim (singleminded protein); GAF, cGMP-stimulated phosphodiesterase, Anabaena adenylate cyclases and Escherichia coli FhIA; PHY, phytochrome; PRD, PAS-related domain; HKRD, histidine kinase—related domain. (Figure of A. Fehér)

7.2.1.2 Functioning of phytochromes

Phytochromes exist in two spectroscopically and functionally different forms which are interconvertible (3). Phytochromes in the dark are in the Pr conformation that is biologically inactive. This form has its absorption maximum at 680 nm (Pr= red-absorbing phytochrome). When it absorbs red light, its conformation changes and the phytochrome takes the Pfr form which has the absorption maximum at 730 nm (far-red light) and is biologically active (triggers signal transduction) (Fig. 7.2.). When the Pfr form absorbs far-red light, it is converted back to the inactive Pr form (photoconversion). The Pfr form can also spontaneously convert to Pr in prolonged darkness (dark conversion), therefore, in the morning, most phytochrome molecules have the Pr conformation.

The absorption spectra of the two forms overlap (Fig. 7.2). It means that neither the red nor the farred illumination result in 100% conversion. Depending on light composition (the ratio of red and farred spectra) a defined equilibrium of Pr/Pfr is established (photostationary state) (3). For example, following pure far-red irradiation, app. 2-3% active Pfr is formed beside the 97-98% Pr, and after pure red-light illumination only 85% is in the active Pfr conformation since 15% is always back converted to Pr. In summary, the ratio of the red and far-red spectrum of the light determines the ratio of the Pfr form in all phytochrome molecules (Pfr/Ptotal). The sensitivity threshold of a given biological process towards Pfr/Ptot, what determines the plant's response to a given light composition.

7.2.1.3 Types of phytochromes

In the model plant *Arabidopsis thaliana*, the phytochrome family consists of five members designated as PHYTOCHROME A, B, C, D, E (PHYA-PHYE) (1). They are classified as Type I (PHYA) and Type II (PHYB-PHYE) phytochromes based on their light stability: The Type I phytochrome PHYA has a high level only

in dark-grown plants/seedlings where it senses very low light intensities and is rapidly decomposed in normal light (5); Type II phytochromes are the light-stable dominant red-light receptors.



Figure 7.2. Wavelength-dependent functioning of phytochromes. A) Absorption spectra of the two conformers -Pr and Pfr- of phytochromes. B) The Pfr/Ptot ratio at different irradiation wavelengths. C) The light-mediated interconversion of Pr and Pfr forms and the isomerisation of the bilin chromophore. (6, with permission from the author).

7.2.1.4 Inactivation of phytochromes

In dark-grown etiolated seedlings, PHYA is the major photoreceptor giving 85% of all phytochromes present. When it is activated, it switches on many genes that ensure plant development and survival when the growing seedling breaks out to sunlight. In light, the degradation of PHYA molecules takes place to remove the active PHYA photoreceptor, which is not further required. This degradation is governed by the COP1 E3 ubiquitin ligase/26S proteasome system (3,7).

In light, PHYB is the major phytochrome molecule (40%). It is stable in light. At the end of the day, almost all PHYB is in the Pfr active form. Their inactivation during the night is mainly due to the spontaneous dark conversion (8). It is the consequence of the lower thermodynamic stability of the Pfr than the Pr form of PHYB. The halftime of this molecular relaxation is app. 60 minutes.

7.2.1.5 Signalling from phytochromes

Phytochromes mediate light signals in two ways: via protein-protein interactions of their N-terminal region and via protein phosphorylation due to the serine/threonine kinase activity of the C-terminal region (3). There are only a few known substrates phosphorylated by phytochromes including the blue light receptor cryptochromes, the PHYTOCHROME KINASE SUBSTRATE 1 (PKS1) protein, and the PHYTOCHROME INTERACTING FACTOR 3 (PIF3) transcription factor (3,9).

Many members of phytochrome signal transduction pathways were identified by investigating Arabidopsis photomorphogenesis mutants (1,3). The hypocotyl of Arabidopsis seedlings developing in

dark elongates, it has a hook at the tip, the cotyledons are closed and whitish. This skotomorphogenic (skoto = darkness) development ensures the proper development of the seedling while it is under the soil where the light is limited and from where it needs to break out rapidly. In light, the seedling stops elongation, its hypocotyl is widening, the hook is straightened out, the cotyledons open and get green to harvest light energy.

Mutation in negative elements of phytochrome signalling results in seedlings growing under dark as they would grow in the light (constitutive photomorphogenesis) (3). These skotomorphogenic factors include the PHYTOCHROME INTERACTING FACTOR transcription factors, and those proteins that are responsible for the degradation photomorphogenesis-promoting factors (see below) in the dark such as the members of the E3 ubiquitin ligase complex: COP1, DET (DEETIOLATED) and FUS (FUSCA) (10).

Missing of the function of the positive elements of the signal transduction obviously cause the opposite (3): due to insensitivity towards light, the mutants continue skotomorphogenic development even in light. This group of mutants identified the phytochrome receptors themselves and those transcription factors that are required for light-activated gene expression and photomorphogenesis such as HY5 (ELONGATED HYPOCOTYL 5), HYH (HY5 HOMOLOG), LAF1 (LONG AFTER FAR-RED 1), HFR1 (LONG HYPOCOTYL IN FAR-RED 1) etc.

Skoto- and photomorphogenic development is mutually exclusive such as the activity of the transcription factors regulating one or the other. The switch between these two developmental pathways as seedlings break out to light is mediated by phytochromes (11) (Fig. 7.3.). The regulation can be briefly described as follows (1,11) (Fig. 7.3.). In dark, the phytochromes are inactive and reside in the cytoplasm as Pr form. In the nucleus, the skotomorphogenesis-promoting PIF transcription factor is active, while those regulating light-activated gene expression are actively degraded by the COP1 E3 ubiquitin ligase/proteasome pathway (10). When the seedling absorbs enough light, the phytochrome is converted into Pfr form that enters the nucleus, interacts with the PIF factor that gets phosphorylated and degraded (12). Meanwhile, also due to the activation of the blue light receptor cryptochromes (see below), the COP1 protein is excluded from the nucleus to the cytoplasm preventing the degradation of the HY5, HYH, LAF1 and HFR transcription factors (10), which can thus accumulate and activate the genes of photomorphogenic development (and repress of those of skotomorphogenesis).



Figure 7.3. Simplified model of the regulation of skotomorphogenesis and photomorphogenesis in dark (A) and light (B). See the text for details. (6, with permission from the author).

7.2.1.6 Classification of phytochrome responses

Early investigations of the effects of red/far-red light on plant development resulted in the classification of plant responses into three categories based on the triggering light dose/intensity (3): 1) very low fluence response (VLFR), 2) low fluence response (LFR), high irradiance response (HIR) (Fig. 7.4.).

VLFR is evoked by extremely low light intensities that cannot be sensed by any other light receptors just the extremely sensitive PHYA. Since PHYA is light sensitive, these responses include those that take place during the development of plants in the dark at their earliest life stage: induction of germination, switch of skoto- to photomorphogenesis. A further characteristic of VLFR is that it cannot be reversed by far-red light since the Pfr/Ptot threshold of these processes is so low that the 2-3% of Pfr form induced by far-red illumination (see above) overcomes it. The so called "law of reciprocity" apply for VLFR, which means that the response is dependent on the total amount of photons received irrespective of the duration of light treatment.

LFR responses are dependent on light-stable phytochromes like PHYB and include e.g. the lightdependent germination of lettuce seeds, leaf movements etc. These processes can be reversed by farred illumination and follow the law of reciprocity.

HIR responses are induced only by prolonged high light intensities. HIR is independent of the light dose, only the strength of the irradiation what counts. However, short interruptions in the illumination can result in drastically reduced response. Therefore, the law of reciprocity does not apply for HIR. Moreover, this response cannot be reverted by far-red irradiation. Both PHYA and PHYB are capable to mediate HIR. PHYA is involved in HIR in response to far-red light (FR-HIR) during the germination of certain seeds. PHYB mediates R-HIR in response to red light e.g. during the inhibition of hypocotyl elongation by light, the induction of flowering, or anthocyanin production in response to high irradiation etc.



Figure 7.4. Classification of phytochrome responses based on irradiation strength affecting the ratio of Pfr/Ptot. (6, with permission from the author).

7.2.2. The blue light photoreceptors: the cryptochromes and the phototropins

The UVA-blue spectrum of light is very important for plants and is sensed by various types of photoreceptors. Probably the two most important and at the same time the best characterized classes are the cryptochromes and the phototropins (13).

7.2.2.1 The cryptochromes

Cryptochromes have evolutional relationship with bacterial photolyases, which have important roles saving bacterial cells from the consequences (of strong UV-C and UV-B irradiation removing/correcting pyrimidine dimers in the DNA molecules for which the energy is obtained by the absorption of blue light (14). Plant cryptochromes lost this enzyme activity but possess a C-terminal region implicated in signal transduction mediated by protein-protein interactions (Fig. 7.5.). The light is absorbed by a pterin (5,10-methenyltetrahydrofolic acid; MTHF) and a flavin (FAD) chromophore that are non-covalently attached to the N-terminal region of the protein. In response to blue light absorption by the chromophores, the molecule will change conformation exposing its otherwise hidden C-terminal region (15). This will allow the interaction of CRY1 with its protein partners including the COP1 E3 ubiquitin ligase component (10). Due to this interaction, COP1 is removed from the nucleus allowing the accumulation of the transcription factors (e.g. HY5) regulating light responses. As such, CRY1 positively regulates photomorphogenesis. Furthermore, cryptochromes play important roles setting up the circadian clock opening the stomata in light, anthocyanin production, and flowering, etc.



Figure 7.5. The basic structure and homology of plant cryptochromes to bacterial photolyases. (Figure of A. Pécsváradi)

7.2.2.2 The phototropins

The phototropin family have two members in Arabidopsis (PHOTOTROPIN 1, 2; PHOT1, 2). Phototropins are app. 120 kDa light-activated serine/threonine protein kinases (16) (Fig. 7.6.). At the N-terminal region of the protein there are two LOV (Light Oxygen Voltage) domains, which bind flavin mononucleotide (FMN) chromophores. Photoexcitation of the LOV domain results in receptor

autophosphorylation and the start of signal transduction via subsequent phosphorylation of cellular targets.

Phototropins, as indicated by their names, mediate the phototropic response of plants. Phototropism is the light-regulated directional growth of plants: shoot growth towards light (positive phototropism) while roots grow to the opposite direction (negative phototropism) (17). This is due to the activation of auxin transport from the illuminated to the shaded sided of the organ. The differential accumulation of auxin results in differential growth resulting organ banding. Phototropins mediate the response phosphorylating the ABCB auxin transporter and the plasmamembrane H⁺-ATPase. The H⁺-ATPase in response to auxin acidifies the cell wall that is a prerequisite of plant cell elongation. Phototropins also regulate the H⁺-ATPase in stomatal guard cells to mediate the opening of stomata in blue light (13). Moreover, they are involved in the regulation of chloroplast arrangements in response to light and leaf movements.





7.2.3. Sensing UV-B by plants

The light spectrum between 280-320 nm is a strong stress factor that if absorbed by macromolecules can cause serious damages in living cells. Plants living in light are continuously exposed to UV-B as well, especially in high mountains. Therefore, plants have evolved protective mechanisms against UV-B including the production of photoprotective pigments and modifying morphogenesis (short stems, smaller leaves turning away from the irradiation, thick layer of wax, moving chloroplast away from the surface etc.) (18). The photoreceptor responsible for UV-B sensing and triggering the above responses is UVR8 (19). This photoreceptor has no chromophore; specific tryptophan residues absorb the light causing a conformational switch that allows the monomerization of the otherwise multimeric UVR8 in the cytoplasm. The UVR8 monomer can enter the nucleus and activate the HY5 transcription factor that is responsible for the UV-B response in addition to its role in photomorphogenesis under visible light (see above) (20).



- 1. Light carries various information about the environment surrounding the plants such as the time of the day, the season, population density, direction of light, degree of shadow etc. Plants have various photoreceptors that are capable to sense many parameters of light including wavelength, intensity, quality, direction, periodicity etc.
- 2. Phytochromes (PHY) are the red-light photoreceptors of plants that exist in tow interconvertible conformations: the red-light absorbing inactive PR, and the far-red light absorbing active Pfr conformers. The ratio of the Pfr/Pr forms defines the plant's response towards the given light composition.
- 3. Phytochromes binds a bilin chromophore and operates as dimers. Light induces isomerization of the chromophores that is translated into a conformational switch in the protein dimer. Signal is transduced via protein-protein interaction of the N-terminal region as well as protein phosphorylation by the C-terminal kinase domain.

- 4. Thy PHYA receptor is light labile and has its role at very low light intensities such as germination and seedling growth underground in the dark. The other phytochromes (PHYB-PHYE) are light stable and regulate various aspects of plant development in light either dependent on light dose or irradiation strength.
- 5. Phytochromes regulate the switch between skoto- and photomorphogenesis (development in the dark and light, respectively) via influencing the stability of transcription factors.
- 6. Blue light is sensed by two main types of photoreceptors, the cryptochromes and the phototropins.
- 7. Cryptochromes have no biochemical activity but exerts their signal transduction task by protein-protein interactions following a light-induced conformational change exposing their C-terminus. These photoreceptors positively regulate photomorphogenesis preventing the degradation of transcription factors governing light responses.
- 8. Phototropins are light-activated protein kinases. Following autophosphorylation, they can phosphorylate cellular proteins mediating light responses such as directional growth towards/from light (phototropism), stomatal opening, etc.
- 9. UV-B is deleterious for plants and induces defence/adaptation mechanisms. This irradiation is sensed by tryptophan amino acids of the UVR8 protein that in response enter the nucleus and activates transcription factors.

Questions

- 1. Why light is important for plants?
- 2. What segments of the light spectrum plants can sense?
- 3. How phytochromes function?
- 4. What is the difference in the functioning of the two types of blue light receptors?
- 5. Why and how plants sense UV-B radiation?

Questions to discuss

1. How light parameters can influence the yield of crops?

Suggested reading

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Chapter 8. The vegetative growth and development of plants

This chapter discusses the specificities of plant growth and development. After defining the basic terms, the morphogenesis of shoots and roots are introduced. More detailed sections deal with the apical meristems producing shoots and roots and the control of their maintenance. The main endogenous factors determining the forming of the plant body, leaves and roots are briefly surveyed. The chapter explores what kind of regulatory mechanisms ensure that plants can grow continuously new leaves and roots even if they live for a several years or hundreds or thousands of years.

Learning goals:

- > Knowledge:
- The students know to distinguish among growth, differentiation and development
- The students know the specificities of plant growth
- The students are able to review the main processes building up post-embryonically the plant body
- The students know the significance and functions of plant meristems
- The students can understand and compare determinate and indeterminate growth
- > Abilities:
- The students can use the obtained knowledge to recognise plant species with determinate/indeterminate growth patterns
- The students can recognise growth problems related to vegetative plant development
- > Attitude:
- The students are open to study the molecular regulatory mechanisms behind vegetative plant development
- Autonomy/responsibility
- The students can autonomously recognise how vegetative plant development relates to the human use of various plants
- The students can independently argue why plant vegetative development is so plastic

8.1. Some specificities of plant growth and basic terms of growth and development

In contrary with animals, where the organs develop embryonically, the development of plants is mainly postembryonic. One of the specificity of the plant growth and development that the initiation of organs begins during the embryogenesis, but they are able to produce new organs thorough their whole life [1]. The formation of plant body jointly depends on the growth, development and differentiation.

Growth of plants is a consequence of increase in cell numbers and in cell sizes. Generally, the growth can be defined as irreversible increase in volume and mass, and may be applied to an organism as a whole or to any of its part [2]. However, growth is not necessarily associated

with increase in dry mass; there are several exceptions. For example, the dry mass of a seedling can be lower than dry mass of the seed, because plant cells may be up to 95% water by weight [3]. Formation of cells, tissues and organs with specialized structures and function is called differentiation. The differentiation usually accompanies growth and, because of the specialization results in "new quality", it often can be regarded as a qualitative change. If differentiation does not occur, the result of the proliferation would be a set of cells with no or little specific function and coordination [2]. The change in form when growth and differentiation is combined is the development. In the frame of Molecular Plant Biology practical course sterile plant tissue cultures will be used to observe formation of mostly undifferentiated cell mass called callus, and also to investigate the endogenous factors determining the differentiation and developing of new organs. The phenomenon of the overall form of a whole plant is the morphogenesis, which involves growth (three-dimensional extension of axes and lateral structures) and differentiation. The activity and interaction of cells are driven by the genetic program and modified by environmental conditions.

The specific regions where cells proliferate, expand and differentiate into new tissues, are localized centres of cell divisions called meristems [1]. Apical meristems found at the tips of stems and roots (shoot apical meristem = SAM, root apical meristem = RAM) are responsible for growing of these vegetative organs. The plant meristems contain cells that, similarly to the animal stem-cells, possess high developmental capacity. The stem-cells in vegetative meristems divide and provide new cells continuously, but they even regenerate themselves so future new tissues can be produced from them. One of the daughter cells may keep the stem cell identity after the cell division, the other gives rise to a file of cells which differentiate into one of the specified cell types [3]. In meristems the number of cells is rather constant, the cell division and differentiation are balanced. Interestingly, the apical meristems have in their centre a population of cells in which the rate of cell division is very low, thus the DNA damages and mutations are minimized. The consequence of this is that even in very long-lived plants such bristlecone pine, where a meristem may survive for 4-5000 years, cells continue to divide accurately throughout their life span [3].

8.2. Shoot morphogenesis

The plant structure is modular. The structural modules of shoots are termed phytomers [3]. Typically, a phytomer comprises a node with an associated leaf, axillary bud and internode segment (Fig. 8.1.). The shoot apical meristem (SAM) produce phytomers repeatedly. SAMs are usually indeterminate, it means that they sustain the capacity to make new leaf and lateral (axillary) bud primordia (which have the potential to form new branches). If the shoot apex terminates in an inflorescence and fruits, the growth is limited, that is called to determinate growth. Such can be observed at some species or varieties, e.g." bushy" tomatoes [3].

The vegetative shoot consists of the stem and leaves. Several regulatory mechanisms, transcription factors and other proteins were identified to be involved in the maintenance of the SAM. Investigation of mutants revealed that a number of proteins are essential for normal functioning of SAM. The proteins, often named after the identified phenotype of the *Arabidopsis* mutants, can be classified into three big categories [5]. One class of genes is required for the establishment and maintenance of the indeterminate central zone of

meristems. It includes several homeodomain-containing transcription factors, e.g. KNOTTED (in Arabidopsis KNAT1), SHOOTMERISTEMLESS (STM), WUSCHEL (WUS). A second class of genes promote cell differentiation in organ primordia (e.g. CLAVATA1 = CLV1, which is a protein kinase). A third class regulate local proliferation of cells in developing organs, for example the PHANTASTICA (PHAN) transcription factor in the upper (adaxial) cell layers of leaves [5].

The regulatory genes that are essential in establishment and maintenance of SAM are expressed in specific domains of meristem. The *WUS* gene is transcribed in the rarely dividing cells of organisation centre at the base of SAM [6]. The WUS protein increase the sensitivity of cells for cytokinin hormone [7]. Around this region express the *SHOOTMERISTEMLESS* transcription factor genes. The WUS and STM proteins are responsible for maintaining the dividing capacity of these cells. Because their differentiation is hampered, they can be regarded as tissue initials (stem-cells). The border between the SAM and the developing leaf primordia are determined by the CUP-SHAPED COTYLEDON (CUC1, CUC2) transcription factors [5]. The CUC and STM transcription factors control of each other's expression and terminate the zones of proliferation and differentiation [8]. The role of STM and the related KNOX transcription factors is the maintenance of synthesis of cytokinins and decreasing the level of gibberellins, thus blocking the growth of leaf primordia.



Fig. 8.1. The plant body has a modular structure. Shoot and root apical meristems produce the phytomers (shoot modules) and root modules, respectively, repeatedly. Stems grow by internode extension. From [3] with permission.

The molecular mechanism responsible for maintaining the WUS gene expression on a relatively constant level is a negative feedback loop with involvement of CLAVATA1-3 (CLV) proteins [9, 10]. The loss-of-function *clavata* mutants have larger SAM, indicating that they are negative regulatory components in determination of SAM size. The CLV1 is a receptor kinase that will be activated by binding the small CVL3 peptide ligand and through a signalling cascade it inhibits the expression of *WUS* gene in the central zone of SAM. The *CLV3* gene is expressed also in the central zone and its transcription is induced by the WUS. If the level of the *WUS* transcript decreases, the expression of *CLV3* will be lowered and results in the increase of the *WUS* gene expression. The elevated WUS level triggers the decrease of its own transcription due to this feed-back loop [10].

8.3. Formation of leaves

Leaf primordia generally form on the flank of shoot apical meristem [3]. SAM produces leaves at regular intervals and in predictable arrangements (phyllotactic pattern). The main types of the characteristic geometry of leaf position (phyllotaxy) are the alternate and spiral when a single leaf is arising from a node, the paired leaves develop oppositely and the position of four or more leaves in one node is usually whorled. The main factor in determining the site of leaf formation is the auxin [11]. Treatment of SAM by low auxin concentration or blocking the auxin transport change the phyllotaxy. The leaf primordia initiate at the local auxin maxima. These auxin maxima are forming due to auxin-regulated asymmetric localization and stability of the PIN1 auxin transporters.

The shape and size of the leaves are determined by different processes such as flattening, orientation and outgrowth of the lamina [3]. Several molecular mechanisms of leaf development are already known. In Arabidopsis, the development of leaf lamina is connected with the adaxial/abaxial polarity which is regulated by antagonistic interactions between different transcription factors. Adaxial cell identity is specified by HD-ZIP III and AS, while the abaxial cell fate by the so-called KANADI and YABBY transcription factors. Among the other elements involved in leaf's planar form auxin response factors and miRNAs were identified too [3].

8.4. Growth and differentiation of roots

Four different zones can be distinguished longitudinally according to the developmental stages and histological characters of cells on the tip of primary root: root cap, zone of cell division (proximal meristem), zone of elongation and zone of maturation (Fig. 8.2.). The root apical meristem produces the cells of the root cap and also cells which differentiate to fulfil specific function. The file of cells with similar origin building up the tissues of roots can be observed microscopically especially on a young root (Fig. 8.2.).

The tip of root is covered and protected by the root cap. Between the root cap and proximal meristem can be found the quiescent centre, that has cells with low dividing capacity [12]. For the maintenance of the root apical meristem (RAM) the WUS-homologue WOX5 transcription factor is responsible. The *WOX5* gene expresses in the quiescent centre, but affect in the neighbouring cells inhibiting their differentiation [13]. Similar molecular mechanisms support the maintenance of the RAM than in SAM. The key component in the development of primary and lateral roots is the auxin. The movement of auxin in the tip of the primary root has a

specific pattern The auxin is transported from the shoot into the root and can be found in high concentration in the proximal meristem (Fig. 8.3). Among the identified very important proteins determining the identity and differentiation of Arabidopsis root cells can be found e.g. PLETHORA (PLT1 and PLT2), ARABIDOPSIS CRINKLY4 (ACR4), CLE44, SCARECROW (SCR) and SHORTROOT (SHR) [3, 5].



Fig. 8.2. The main structural element, zones and tissues of onion root tips. The root apical meristem (RAM) and the cell lines originated from it are highlighted. From [3] with permission.



Fig. 8.3. The polar auxin transport in the tip of Arabidopsis primary root. Auxin is transported from the shoot apex to the root cap through the vascular tissues and moves up in the endodermis. From [3] with permission.

Summary

- 1. The initiation of the plant organs begins during the embryogenesis, but they are able to produce new organs thorough their whole life. The formation of plant body jointly depends on the growth, differentiation and development.
- 2. The growth is defined as irreversible increase in volume and mass. It is a consequence of cell division and enlargement. Formation of cells, tissues and organs with specialized structures and function is called differentiation. Morphogenesis is the forming of the whole plant and involves growth and differentiation. The morphogenesis is driven by the genetic program and modified by environmental conditions.
- 3. Cells in plant meristems possess high developmental capacity. Shoot apical meristem (SAM) and root apical meristem (RAM) are found at the tips of stems and roots, respectively.
- 4. The plant body has a modular structure. The module produced by shoot apical meristem is called phytomer and comprises a node with an associated leaf, axillary bud and the internode segment.
- 5. In meristems the number of cells is rather constant, the cell division and differentiation are balanced. Stem-cells in vegetative meristems divide and provide new cells that will specialize, but they also regenerate themselves.
- 6. A negative feedback loop ensures the maintenance of the function and size of SAM. The CLAVATA1 (CLV1) receptor kinase is activated by the small CVL3 peptide and inhibits the expression of the *WUSCHEL (WUS)* gene in the central zone of SAM by inducing a signalling cascade. If the level of *WUS* transcript enhances, it increases the expression of *CLV3*, thus decreasing later its own transcription.
- 7. SAM produces leaves at regular intervals and in predictable arrangements, the phenomenon is the phyllotaxy. The leaf primordia initiate at the local auxin maxima that are forming due to auxin-regulated asymmetric localization and stability of the PIN1 auxin transporters. Development of the adaxial/abaxial polarity of leaves is regulated by antagonistic interactions between different transcription factors.
- 8. Four different zones can be distinguished longitudinally on the tip of primary root: root cap, zones of cell division (proximal meristem), elongation and maturation. The *WUS*-homologue *WOX5* transcription factor gene expresses in the quiescent centre, but inhibit the differentiation of the neighbouring cells. A similar molecular mechanism supports the maintenance of the root apical meristem (RAM) than that in SAM. The key component in the development of primary and lateral roots is the auxin. The auxin moves from the shoot into the tip of the primary root, but direction will turn and moves up in the endodermis.

Questions

- 1. What is the difference between growth, differentiation and development?
- 2. What does it mean that the shoot has a modular structure?
- 3. What are common in meristematic tissues and in their maintenance?
- 4. Which plant hormone is the most important in plant morphogenesis?
- 5. How develop a leaf?
- 6. Which longitudinal regions or zones can be identified in roots?

Questions to discuss

- 1. What kind of environmental factors influence the morphogenesis of plants?
- 2. Why are important the growth and development of plants for the mankind?

Suggested reading

- The seed plant body plan II. Form and function of organ systems. In: Jones R, Ougham H, Thomas H, Waaland S (Eds) The molecular life of plants. Wiley-Blackwell, American Society of Plant Biologists, 2013. pp. 27-32.
- Growth and development. In: L. Taiz and E. Zeiger (Eds), Plant physiology. Sinauer Associates Inc., Publishers Sunderland, Massachusetts, U.S.A., 5th Edition, 2014., pp. 339-374.

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Chapter 9. Flowering

Flowering is a major stage in the plant's life cycle marking the transition from vegetative to the reproductive development that takes place primarily in the shoot meristem. Precise timing of this transition guarantees reproductive success and, therefore, is tightly regulated by developmental and environmental constrains. In this chapter, the developmental and molecular background of flowering time, flower meristem identity, and flower organ development will be discussed in a comprehensive way.

Learning goals:

> Knowledge:

- The students understand the vegetative-reproductive developmental transition in plants
- The students are able to compare the shoot, the inflorescence, and the flower meristems
- The students understand how inflorescence structure is regulated
- The students can compare the photoperiodic, vernalisation, and age-dependent pathways of flowering time regulation
- The students can explain the importance of integrating environmental and developmental signals during the decision to flower
- The students know the extended ABC model of flower organ development
- The students can describe the regulation cascade integrating environmental signals with flower development
- > Abilities:
- The students can use the obtained knowledge to recognise when and why a plant starts flowering
- The students can explain various flower types beside the learned examples
- > Attitude:
- The students are open to investigate various flowers
- The students see the beauty of flowers from a new perspective understanding the developmental mechanisms behind them
- Autonomy/responsibility
- The students can autonomously explain for others how flowers develop and why they are so variable
- The students can independently argue about the importance of flowering time and pollination to ensure plant yield in agriculture

9.1. The vegetative-to-reproductive transition

During vegetative growth, the shoot meristem repeatedly produces vegetative phytomer units consisted of a node, an internode, a leaf, and an axillary meristem. This is an indeterminate process that ensure the continuous growth of the shoot. Following the decision to flower, the shoot meristem changes fate and become an inflorescence meristem (or a flower meristem if the plant develops only one flower at the shoot apex). The inflorescence meristem still growth in an indeterminate way, but instead of vegetative leaves, it produced flower meristems at its flanks (or, with a certain frequency, bracts with axillary inflorescence meristems). Arabidopsis is an appropriate model to investigate this

transition, since during vegetative growth it forms only a rosette of leaves characterized by short internodes, while during the reproductive transition inflorescence stems with long internodes, bracts, and flowers are formed.



Figure 9.1. Comparison of the vegetative shoot and the flower. Flower is a modified shoot producing only four phytomer units, each with short internode and different organ, but no axillary meristem. (Figure of A. Fehér)

The flower meristem has a determinate growth pattern, since it stops growing after producing four nodes having the various flower organs. It has special characteristics: every node has a different organ on it, has no axillary meristem, and the organs are arranged in whorls. Nevertheless, both the inflorescence and the flower are modified shoots (Fig. 9.1). The shoot meristem must go through well-defined gene expression changes to acquire new fates and form new organs during reproductive growth. The most important genes having role in this process could be identified investigating appropriate Arabidopsis mutants.

9.1.1 The flower meristem identity genes

Mutations in genes determining flower meristem identity were expected to convert flowers back to inflorescences or shoots. This was indeed the case. Two such genes were discovered: *LEAFY (LFY)* and *APETALA1 (AP1)* both coding for transcription factors (1,2). In case of the *leafy* mutation instead of the flowers leaves or false flowers (with leaf-like structures instead of flower organs) are produced. The *apetala1* mutation converts the flowers back to inflorescence-like structures (secondary, tertiary etc. flowers within flowers). If both genes are mutant in the same plant, then flower formation is fully blocked, and vegetative shoot develops instead of the inflorescence. LFY and AP1 have non-overlapping essential function during the establishment of flower meristems (3). These two transcription factors are synergistic; they promote each other's production that helps to maintain meristem identity.

9.1.2. The inflorescence meristem and its structure

Where the shoot develops only one flower at the tip, the whole shoot meristem is converted to flower meristem and flower formation results in the determination of shoot growth. Where inflorescence develops, the meristem at the shoot apex maintains its indeterminate growth besides forming flowers. Arabidopsis develops in this way. However, there is a mutation that converts the indeterminate inflorescence meristem of Arabidopsis into a determinate flower meristem. In this mutant, there is only one terminal flower on each stem and the gene identified by this mutation was named as TERMINAL *FLOWER 1 (TFL1)* (4). Interestingly, the same phenotype is the result of the overexpression of LFY and AP1 in the inflorescence meristem! Moreover, the overexpression of TFL1 in the meristem has the same consequences as the *lfy* and *ap1*mutations. These observations can be explained if the TFL1 and the LFY/AP1 proteins are antagonistic and inhibits each other's production (5). It is indeed the case: in the middle of the inflorescence meristem the *TFL1* gene is active and TFL1 blocks the expression of *LFY* and *AP1* (Fig. 9.2). Therefore, there is no determinate flower development and the

inflorescence continues to grow. However, at the flanks of the meristem, LFY and AP1 are produced and inhibit TFL1 expression resulting in the formation of flowers and the cessation of growth (Fig. 9.2).



Figure 9.2. Determination of inflorescence and flower meristem identity by the antagonistic protein factors *TERMINAL FLOWER 1 (TFL1)* responsible for indeterminate inflorescence growth and LEAFY (LFY), APETALA1 (AP1) defining flower meristem identity. (Figure of A. Fehér)

The antagonism of TFL1 (indeterminate growth) and LFY/AP1 (determinate growth) defines the structure of the inflorescence depending on the spatial and temporal expression pattern of these factors (Fig. 9.3) (6). The arrangement of gene expression domains of TFL1 and LFY/AP1 that was described above for Arabidopsis (TFL1 in the middle, LFY/AP1 in the flanks) results in a raceme-type inflorescence, while the opposite, results in a cyme (Fig. 9.3). If the expression of these factors is separated by time rather space and first all meristem expresses TFL1 producing a branching inflorescence and then all meristem starts to express LFY/AP1 and produce flower, the inflorescence will be a panicle (Fig. 9.3).



Figure 9.3. The spatial and temporal expression of the antagonistic regulators TFL1 and LFY (with AP1) in the shoot meristem (above) determines the inflorescence structure (below). (Figure of A. Fehér)

green – vegetative tissue;

red - TFL1 expression, indeterminate growth; yellow - LFY and AP1 expression, determinate flower meristem.

9.1.3 Regulation of flowering time

Timing of flower initiation and development has central importance for plant reproduction. Efficient fertilisation requires synchronous flowering of cross-pollinating plants, synchrony between flowering and pollinator animal activity, synchrony between flowering, seed set, germination and preferential environmental conditions for fertilisation, seed dispersal, and the establishment of the new

generation. Some of the main parameters affecting flowering time, such as photoperiodism, cold treatment, plant age, and the gibberellin hormone will be discussed in this part of the chapter.

9.1.3.1 The flowering integrator factors

The various parameters affecting flowering time needs to be integrated into one response. This is achieved by the so-called flowering integrator factors. These genes were identified by Arabidopsis mutations which delayed flowering under inducing conditions. The genes disrupted by the mutations were isolated and their overexpression was shown to induce flowering under non-inducing conditions. These experiments clearly indicated the central role of these genes in the induction of flowering. Two of the most important flowering integrators are FLOWERING LOCUS T (FT) and SUPPRESSOR OF CONSTANS 1 (SOC1) (7). SOC1 is a transcription factor but FT is not. However, FT was shown to interact with and activate the transcription factor FLOWERING LOCUS D (FD). Targets of SOC1 and FT/FD are the genes coding for LFY and AP1, respectively. Thus, environmental and endogenous signals switch on flower meristem identity genes via the activation of flowering integration factors.

9.1.3.2 Photoperiodic regulation of flowering

The length of day and night is equal at the Equator, but towards the poles day length increases during the summer and decreases during the winter. Plants developing at the various geographical latitudes accommodated to these conditions adjusting their life cycle, including flowering, to the cycle of seasons. Plants can be classified as short-day, long-day or day-neutral plants depending on the day length that is required to induce flowering. The short-day and long-day plants have different critical values of day length and flower if the light period is shorter (short-day plants) or longer (long-day plants) than this threshold. Long-day plants usually flower in the spring and early summer, while short-day plants at the end of summer or during the autumn. However, the photoperiod is an ambiguous signal, since each day/night ratio manifests itself twice a year. Therefore, plants either combine temperature sensing with photoperiodism (see later for vernalisation) or they record the tendency whether the night periods are shortening or lengthening during subsequent days. Moreover, the developmental state can also affect flowering (see also later). A plant can be in a juvenile non-competent state in a given season and in a mature flowering-competent state only in the other.

Although plants are classified according to their day-length requirement for flowering, a series of experiments showed that plants measure the length of the night (dark period) not the day (light period) (Fig. 9.4) (8). At a defined day length, the flowering response is dependent on the critical value of the dark period (Fig. 9.4). However, if the dark period is set, changing the length of the light period does not affect the flowering response (Fig. 9.4). Furthermore, disrupting the dark period by short time of irradiation (few minutes) is enough to cancel the effect of the dark period on flowering, but disrupting the light period by dark has no effect (Fig. 9.4). If the dark period is interrupted by light, the plants restart measuring its length, while the opposite does not take place.



Figure 9.4. Effect day- and night length on the photoperiodic regulation of flower initiation. The length of the night exceeding a critical value promotes (short day plants) or inhibits (long day plants) flowering. Interruption of the dark period by a light pulse resets the start of measurement. Interruption of the light period by dark has no effect. (Figure of A. Fehér)

Based on the observation that the flowering of short-day plants can be delayed by a short light pulse applied during the night, the researches could investigate the effect of several light parameters on flower induction (9). They observed that:

- the effect of light depends on its wavelength
- the spectrum of the effective wavelengths overlaps with the absorption spectrum of phytochromes
- if the dark-interrupting light is red, flowering is delayed, but far-red light is inefficient in this regard
- if several red and far-red pulses are applied alternately the last pulse is the decisive
- the efficiency of the light signal that interrupts the dark period and delays flowering depends on the time of application: it is most effecting around the middle of the dark period.
- if plants are grown under continuous dark, the efficiency of the light pulse cycles with an app. 24h-long period.

From these observations it was concluded that the red/far-red light sensitive photoreceptors, the phytochromes, have role in the measurement of day/night length. The periodicity in the efficiency of the dark-interrupting light suggested that the circadian clock might also be included in the regulation.

The circadian clock is an endogenous time-keeping mechanism of living organisms. It allows the synchronization of physiological and metabolic processes with the exogenous dark-light cycle, which strongly influences the biological activities of the organisms including plants (10). The circadian clock has an app. 24-hour period, and is "free-running", what means that it maintains its rhythmicity even under constitutive environmental conditions (constant light/dark). This endogenous mechanism is

based on multi-component molecular oscillators. These oscillators are transcriptional/translational regulatory loops of transcriptional factors and regulatory proteins. The output is altered gene expression, app. 10% of all Arabidopsis genes exhibit circadian rhythmicity in their expression pattern. The input is light. Although the clock is free-running, it needs light to be precisely adjusted to the changing environmental dark/light cycle as well as to maintain its robustness (the amplitude of the cycles diminishes under constant conditions).

Based on the above observations, Erwin Bünning (1936) established the model of photoperiodic regulation of flowering (9). According to this model, the plant's sensitivity towards a flowering-inducing signal exhibits a circadian rhythmicity and it has its maximum value around sunset. During long days, but not under short days, the sensitivity maximum coincides with the presence of light and this coincidence that is required for flowering initiation in long-day plants or its inhibition in short-day plants ("Bünning hypothesis" or "coincidence model").

During the past decades, molecular genetic studies confirmed the basic hypothesis of this model. In Arabidopsis, a mutation was identified which made the flowering time of this plant independent of the photoperiod. *Arabidopsis thaliana* is a facultative long-day plant species: it has an early flowering phenotype under long days, and a late flowering phenotype under short days. The mutant exhibited late flowering under both conditions and the corresponding gene was named *CONSTANS* (*CO*) (11). Expression of the *CO* gene exhibits circadian rhythmicity with an expression maximum at evening around sunset (12). Therefore, this gene coding for a transcription factor, display the characteristics of the "sensitivity factor" hypothesized by Bünning. But how CO as a transcription factor regulates flowering?

The decision to flower takes place in the shoot meristem (see before). However, CO is expressed in the leaf primarily in the cells around the phloem. It means that the signal that evokes flowering is perceived in the leaf and gets transferred to the shoot apex. If in the *co* mutant background, CO was overexpressed under the control of a meristem specific promoter it could not complement the late flowering phenotype. If the same experiment was repeated using a promoter working in phloem companion cells, the complementation worked. These experiments proved the CO has its function in the leaf and there should be a mobile signal that transfers the information generated by CO from the leaf to the shoot meristem (12). Scientists has long supposed the existence of a mobile molecule that accumulates in leaves and induce flowering in the shoot apex and called this hypothetical hormone as "florigen", after Mikahail Chailakhyan (1937). Early experiments showed that from a plant that was induced to flower, the flowering signal could be transferred by grafting a single induced leaf to a none-induced plant.

Molecular genetic investigations revealed that the target of the CO transcription factor is the *FLOWERING LOCUS T (FT)* gene, one of the flowering integration proteins (see above) (12). Since CO is expressed in leaves, FT is also formed in leaves. However, scientists showed that if the FT protein is marked with a green-fluorescent protein (GFP) and expressed in the phloem companion cells of leaves, the fluorescent FT protein soon accumulates in the shoot meristem. FT in the meristem interacts with the FD transcription factor and initiates the expression of the flower meristem identity genes AP1 and LFY (via SOC1) (Fig. 9.5). Based on this, the *FLOWERING LOCUS T (FT)* protein is the long-searched "florigen"; the mobile factor formed in leaves but triggering the flowering response in the soot meristem.



Figure 9.5. The model of photoperioddependent signalling between the leaf and the shoot meristem. CONSTANS (CO) switches on the expression of the FLOWERING LOCUS T (FT) gene under long-day photoperiod in the leaf. FT is transferred in the phloem to the shoot meristem where it associates with the FLOWERING D (FD) transcription factor to trigger the expression of the flower meristem identity factors APETALA 1 (AP1) and LEAFY (LFY). Induction of LFY expression is indirect via the expression of the flowering integrator OF SUPPRESSOR CONSTANS 1 (SOC1) transcription factor. The synergistic AP1 and LFY are antagonistic with TFL1 to establish the inflorescence meristem (see Fig. 9.2). (Figure of A. Fehér)

But why the *FT* gene is switched on by CO only under long days? Why the activity of the CO gene during the dark period of long nights is not sufficient to evoke the flowering response? The answer is in the stability of the CO protein that is regulated by light (Fig. 9.6) (9). During short days, the maximum of CO expression is in the dark. Although CO proteins are produced, those are rapidly degraded in the dark and cannot switch on efficient transcription of FT (Fig. 9.6) (12). Under long days, however, CO protein is already produced in the evening when light is still present. Light prevents the degradation of CO that switches on FT gene expression and thus FT protein accumulates at a sufficient level to get transported to the shoot meristem and trigger the flowering response (Fig. 9.6) (12). Light exerts its effect on the CO protein via the PhyA and Cry1 photoreceptors at the evening and PhyB in the morning.

Among the short-day plants, the flowering regulation of rice (*Oryza sativa*) has been studied in details (13). The rice genome also codes for CO- and FT-like proteins called HEADING DATE 1 (Hd1) and HEADING DATE 3a (Hd3a), respectively. However, the mechanism of regulation differs between Arabidopsis and rice. In rice the CO homologue protein Hd1 is converted by light from an inducer to a repressor. Thus, Hd1 activates Hd3a (FT homologue) expression during the dark evenings under short days but inhibits it at long days due to the presence of light in the evening period when the Hd1 gene is transcribed.

9.1.3.3 Regulation of flowering by vernalisation

Vernalisation is the process during which extended cold (-2 to +10 °C) treatment releases the inhibition of flowering (14). Vernalisation takes place in the shoot meristem and requires active metabolism and cell division. The effect of vernalisation on flowering differs from that of the photoperiods: vernalisation does not induce the flowering response only makes the plant competent to flower under appropriate inducing conditions. Vernalisation is often coupled to the requirement for long-day conditions to induce flowering, since this ensures that flowering takes place following the winter period during spring or early summer. This also means that the effect of vernalisation remains for long time. In some plants, e.g. in overwintering Arabidopsis ecotypes, it means that the effect of vernalisation is maintained during the whole remaining life period of the plant.



Figure 9.6. Photoperiodic regulation of flowering by the circadian clock-regulated expression and lightregulated protein stability of CONSTANS (CO) switching on the expression of the flowering integrator *FLOWERING LOCUS T (FT)* gene. (Figure of A. Fehér)

The gene expression pattern of the shoot meristem of Arabidopsis ecotypes requiring or not vernalisation for flowering were compared. Among others, a characteristic difference was observed in the expression of the *FLOWERING LOCUS C (FLC)* gene that was only expressed in those ecotypes the flowering of which is prevented or strongly delayed in the absence of vernalisation. The FLC protein proved to be an efficient repressor of flowering (15). This transcription factor prevents the expression of the *FT* gene in the leaf and the *FD* and *SOC1* genes in the shoot meristem (Fig. 9.7). The extensive cold experienced by these plants during winter switches off the *FLC* gene permanently. This is achieved by the modification of the chromatin at the *FLC* locus. In response to cold a specific enzyme complex gets activated, which reorganises the chromatin (the DNA-histone complex) around the promoter and coding regions of the FLC gene. This reorganisation is based on the post-translational modification (deacetylation and methylation) of specific histone residues affecting the chromatin structure (16). As a result, the chromatin at the FLC locus gets densely packed preventing the binding and functioning of transcriptional regulators responsible for FLC transcription.

Vernalisation requirement evolved several times independently in plants growing in the temperate geographical regions. E.g. in winter cereals, completely different proteins play role in the process including VERNALISATION 2 (VRN2) as the flowering repressor (14).



Figure 9.7. The model, how vernalisation regulates flowering competence. FLOWERING LOCUS C (FLC) protein is a potent repressor of the flowering integrator genes FLOWERING LOCUS T (FT) and SUPPRESSOR OF CONSTANS 1 (SOC1). Cold results in the remodelling of the chromatin at the FLC locus preventing FLC expression and releasing FT and SOC1 from repression. (Figure of A. Fehér)

9.1.3.4 Developmental regulation of flowering

In most of the cases, plants flower after a considerable time of vegetative development. In perennial plants, such as trees, it can mean several years or even decades. The life period preceding flowering competence is called as the juvenile phase (17). However, the absence of flowering is not an absolute marker of juvenility since flowering of mature plants depends on many other conditions. In some plants, maturity is marked by morphological changes such as leaf shape, altered phyllotaxy, the development of thorns etc. One- or two-year plants often flower after reaching a given number of leaves, or more exactly producing a given number of phytomers (nodes) (18). E.g. tobacco flowers after developing 41 nodes; if 5 nodes are removed from the top, exactly 5 is reproduced before flowering. Reaching the 41 nodes, the uppermost phytomers get determined to flower; if they are removed and rooted, they immediately start to produce flowers without further vegetative growth. But how plants can count the number of their leaves. Experiments indicate that the amount of sugars produced by the leaves is a possible signal (19). Obviously, more leaf can produce more sugars. The increased sugar level affects a molecular mechanism that is responsible for the juvenile/mature transition. The core of this machinery is based on two antagonistic transcription factor(s) (Fig. 9.8): APETALA2 (AP2) maintaining juvenility, and SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 3,4, and 5 (SPL3,4,5) promoting maturity and flowering (17). The expression of AP2 continuously decreasing while that of the SPL factors continuously increasing during the life time of Arabidopsis. SPL expression reaches its maximal level when Arabidopsis starts to flower. The targets of SPL3 are the flowering integrators FT, SOC1 and the flower meristem identity genes LFY and AP1. The level of SPL3 is kept low during the juvenile phase by a microRNA factor (miRNA156) the level of which is decreasing by increased sugar production.

The biological age, similarly to vernalisation, does not directly induce flowering, only makes the plant competent to flower under appropriate conditions.



Figure 9.8. Hypothetical model of the regulation of flowering competence by plant age. Green – juvenility; yellow – maturity. Blunt arrow - inhibition, pointed arrow - promotion. (Figure of A. Fehér)

9.1.3.5 Gibberellin, as flowering hormone and the autonomous pathway of flowering

Among plant hormones, gibberellin (GA) has a clear effect on the timing of flowering (20). In Arabidopsis, GA level increases at the time of flower formation. Moreover, exogenous gibberellin accelerates Arabidopsis flowering under none-inductive short-day conditions. Under long days, gibberellin-less or gibberellin signal transduction Arabidopsis mutants cannot flower. Gibberellin is also linked to vernalisation in a way. Exogenous gibberellin makes possible in certain plants to overcome the requirement for vernalisation. However, this effect of GA is independent of the level of FLC. Therefore, the vernalisation and GA-related pathways are parallel. GA has been shown to induce the expression of the SPL3, SOC1, and LFY transcription factors implicated in flowering (see above).

In Arabidopsis several further mutations were identified that affect flowering time. These all were shown to affect basic molecular processes, which influence FLC gene expression or the level of the FLC protein. Although these functions do not represent a regulatory pathway, together they are often referred as the "autonomous pathway" of flowering (21).

9.2 Flower morphogenesis

The flower meristem of Arabidopsis produces four different organs in the four whorls of the flower: sepals, petals, anthers and carpel. It indicates that different morphogenetic pathways and gene expression programs are operating in the different whorls. How the flower meristem can govern this variety of programs?

9.2.1. The ABC model of flower morphogenesis

The scientists searching an answer for this question were looking for Arabidopsis mutants where flowering was not affected only flower organ formation. Three classes of mutants could be identified (22): in the "A" class, the mutants had neither sepals nor petals but all four whorls had anthers or carpels (the two mutants were named *apetala1* and *apetala2*; note: the corresponding genes APETALA 1 and APETALA 2 has other functions as well as it was already discussed); in the "B" class, the mutant

flowers had neither petals nor anthers, only sepals and carpels in all whorls (two mutants falling into this category were named as *apetala3* and *pistillata*); in the "C" class there was only one mutant that had flowers having only sepals and petals in all whorls (no anthers, no carpels, therefore, it got the name *agamous*). In these mutants, the formation of given flower organs was not prevented, but a given type of organ was converted into another. This type of mutation is called "homeotic".

The above findings indicate that only three classes of mutations exist for four flower organs. This contradiction could be resolved by establishing the so called "ABC model" of flower development (22,23). The model is based on the assumption that the genes responsible for the "A", "B" and "C" functions corresponding to the "A", "B", and "C" mutations, are expressed in partially overlapping fashion in the four whorls (Fig. 9.9). In the first whorl, only genes of the "A" function (*APETALA 1* and 2; *AP1* and *AP2*) are expressed and govern sepal development; in the second one, in addition to the "A" function genes, those of the "B" function (*APETALA 3* and *PISTILLATA*; AP3 and PI) are also expressed and there petals develop; in the third one, the genes of function "B" and that of function "C" (*AGAMOUS*; *AG*) are transcribed leading to anther initiation; and finally, in the fourth whorl, only the gene of the function "C" (*AG*) is active leading to the formation of carpel (Figure 10).



Figure 9.9. The ABC model of flower organ formation of wild type and the five homeotic flower development mutant Arabidopsis plants forming three classes A, B and C. The ABC mutant classes indicate "functions" required organ development. for flower These "functions" are carried out by organ identity transcription factors. Combinations (overlapping expression) of these factors define organ identity: A = APETALA1 and 2 = sepals and petals; B = APETALA3 and PISTILLATA = petals and anthers; C = AGAMOUS = anthers and carpels. "A" and "C" functions restrict each other's expression. If one is removed the other occupies the whole meristem changing organ identity in the two other whorls. (Figure of A. Fehér)

This model fully explains the phenotypes of the ABC mutants, if one further condition is met (Fig. 9.9): the "A" and "C" function genes must be antagonistic restricting each other's expression. If one of them is removed by mutation, the other function extends to the whole meristem.

One may ask the question, how the same meristem identity factors can regulate the various developmental pathways in the four whorls. The answer is that they have different co-factors in each four whorls modifying their target gene set.

9.2.2. The extended ABC model and the quartet model of flower development

If all five genes involved into the ABC model are mutated in a single plant, instead of real flowers only green pseudo-flowers will develop with leaf-like organs in each whorl. This confirms that the flower organs are modified leaves and the flowers are modified shoots. The flower organ identity homeotic

transcription factors convert leaf development into flower organ development. One can assume that overexpressing these factors in vegetative leaf primordia according to the rules of the ABC model, the primordia will develop into flower organs. However, experiments showed that it is not the case. Other factors are also required for flower organ morphogenesis. These factors were also identified via the characterization of Arabidopsis flower morphogenesis mutants. Arabidopsis has four SEPALATA (SEP1,2,3 and 4) transcription factors with overlapping functions. If all four SEP gene is mutated in one plant pseudo-flowers develop instead of real ones: the ABC factors without the SEP factors are not enough to drive the morphogenesis of the flower (24). This finding is explained by the "quartet model of flower development" (25). This model states that in whorl a guartet of transcription factors forms a complex that regulates the development of the given organ (Fig. 9.10). The SEP factors are and must be present in each quartet since they keep together the functional complexes. This hypothesis was validated by experiments where the flower organ identity transcription factors were overexpressed in vegetative leaf primordia together with a SEP factor and instead of leaves these primordia developed to flower organs (e.g. petals) (26). The SEP factors were included into the extended ABC model as responsible for the "E" function (the "D" function was earlier used for transcription factors determining ovule identity).



Figure 9.10. The quartet model of flower organ development. In each whorl a quartet of transcription factors determine organ identity. SEPALLATA (SEP) transcription factors are required in each quartet besides the transcription factors of the ABC model (see Fig. 9.9). (Figure of A. Fehér)

9.2.3. The validity of the ABC model

Further studies confirmed that the basic mechanisms of the extended ABC model of Arabidopsis are operating in all investigated flower types (27) (Fig 12). The great variability of the morphology of flowers is due to alterations in the temporal and spatial gene expression pattern, the number and novel functions of the homeotic transcription factors and their target genes. One simple example is tulip. The tulip flower has no sepals only petals. In its flower meristem, the "B" function genes are expressed in the first three whorls and therefore in the first whorl instead of sepals also petals will develop (Fig. 9. 12). The complex flower structure of orchids is due to the amplification of the "B" function gene *AP3*. The new AP3 copies have different regional expression patterns in the flower meristem and activate specific target genes regionally defining different sepal and petal types in the two outmost whorls and the fusion of anthers and carpels in the two innermost whorls (Fig. 9.11). In modern roses, the extension of the expression domain of the "A" function in expense of the expression domain pf the "C" function resulted in more petals instead of anthers. The very different flower type of cereals and grasses can also be explained by a variant of the ABC model.



Figure 9.11. Examples for the variations of the ABC model of flower development. Details are discussed in the text. (Figure of A. Fehér)

The AGAMOUS (AG) transcription factor is expressed in the middle of the flower meristem. Beside of its function to regulate anther and carpel development in this region, this factor is responsible for the determinate development of the flower. The WUSCHEL (WUS) homeotic transcription factor regulates the indeterminate growth of the vegetative shoot meristem maintaining the stem cell pool. AG switches off WUS expression in the middle of the meristem after the completion of the development of the carpel-producing fourth whorl of the flower.

9.3. The integrated model for the regulation of flowering time and flower morphogenesis

Based on the above described observations primarily made using Arabidopsis mutants, a regulatory cascade of transcription factors is responsible for the integration of external and endogenous signals leading to flower formation under appropriate conditions. This regulatory cascade is shown in Fig. 9.12.



Figure 9.12. Summarizing model of the regulatory transcription factor cascade integrating flowering time regulation and flower organ development in response to external and endogenous signals.



- 1. Precise timing of vegetative-reproductive transition guarantees reproductive success and, therefore, is tightly regulated by developmental and environmental constrains.
- 2. Following the decision to flower, the shoot meristem changes fate and become an inflorescence meristem. The inflorescence meristem still growth in an indeterminate way, but instead of vegetative leaves, it produced flower meristems at its flanks. Flowers are modified shoots producing only four phytomer units, each with short internode and different organ.
- Inflorescence and flower meristem identity are separated by the antagonistic protein factors TERMINAL FLOWER 1 (TFL1) responsible for indeterminate inflorescence growth and LEAFY (LFY), APETALA1 (AP1) defining flower meristem identity. This antagonism defines the structure of the inflorescence.
- 4. Plants can be classified as short-day, long-day or day-neutral plants depending on the day length that is required to induce flowering. Although plants are classified according to their day-length requirement for flowering, a series of experiments showed that plants measure the length of the night (dark period) not the day (light period).
- 5. The red/far-red light sensitive photoreceptors, the phytochromes, have role in the measurement of day/night length and the circadian clock is also included in the regulation.
- 6. The plant's sensitivity towards a flowering-inducing signal exhibits a circadian rhythmicity and it has its maximum value around sunset. During long days, but not under short days, the sensitivity maximum coincides with the presence of light and this coincidence that regulates (promotes or inhibits) flowering initiation ("Bünning hypothesis" or "coincidence model").

- 7. The decision to flower takes place in the shoot meristem but the photoperiodic signal that evokes flowering is perceived in the leaf. The mobile factor mediating the flowering response between the leaf and the shoot apex was named as "florigen".
- 8. The molecular model of photoperiod-dependent initiation of flowering in Arabidopsis (longday plant): CONSTANS (CO) switches on the expression of the FLOWERING LOCUS T (FT) gene under long-day photoperiod in the leaf. FT is transferred in the phloem to the shoot meristem where it associates with the FLOWERING D (FD) transcription factor to trigger the expression of the flower meristem identity factors. FT protein = florigen.
- 9. Vernalisation is a process in the shoot meristem during which extended cold treatment releases the inhibition of flowering. Vernalisation does not induce the flowering response only makes the plant competent to flower under appropriate inducing conditions. The extensive cold experienced by the plants switches off the flowering repressor FLOWERING LOCUS C (FLC) gene permanently. This is achieved by the modification of the chromatin at the FLC locus.
- 10. In most of the cases, plants flower after a considerable time of vegetative development. The biological age, similarly to vernalisation, does not directly induce flowering, only makes the plant competent to flower under appropriate conditions.
- 11. Among plant hormones, gibberellin (GA) has a clear effect on the timing of flowering.
- 12. The various parameters affecting flowering time needs to be integrated into one response. This is achieved by the so-called flowering integrator transcription factors.
- 13. Flower organ formation is explained by the "ABC model". Three classes (A, B and C) of transcription factors have partly overlapping expression patterns in the four flower whorls. Their specific combination in each whorl defines a given flower organ. According to the "quartet model" of flower development, in addition to the A, B, and C organ identity factors an "E" factor is also required to build up the fully functional and specific "transcription factor quartets" in each whorls.
- 14. The basic mechanisms of the extended ABC model of Arabidopsis are operating in all investigated flower types. The great variability of the morphology of flowers is due to alterations in the temporal and spatial gene expression pattern, the number and novel functions of the homeotic transcription factors and their target genes.

Questions

- 1. What the vegetative-reproductive transition means?
- 2. What discriminates the shoot, inflorescence and flower meristems?
- 3. How flower meristem and inflorescence meristem identities are regulated?
- 4. What kind of conditions influence the flowering time?
- 5. What the photoperiodic regulation of flowering means?
- 6. What is the significance of the photoperiodic regulation of flowering?
- 7. Plants measure the length of the day or the night to take the decision to flower or not?
- 8. What is the "circadian clock"?
- 9. What is the "florigen"?
- 10. What is vernalisation and how does it affect flowering?
- 11. How plant age regulates flowering?
- 12. Which plant hormone has important role in flowering time regulation?

- 13. Explain the extended ABC model of flower morphogenesis!
- 14. What is the essence of the "quartet model" of flower organ development?
- 15. Is the ABC model of flower development valid for other plants in addition to Arabidopsis?

Questions to discuss		
	Questions to discuss	

1. Importance of flowering time in agricultural/horticultural production.

Suggested reading

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Chapter 10. Plant senescence and death

This chapter discusses the plant senescence responses and the level of cell death processes.

Senescence and programmed cell death are processes which are highly regulated and properly ordered and cytological and biochemical changes can occur in order to reuse critical nutrients.

This chapter will discuss the variety of plant senescence phenomena and those factors (external and internal) which contribute to these processes.

Some learning goals:

> Knowledge:

- The students can define senescence and its types in plants
- The students can summarize the role of senescence in plants
- The students can explain what PCD is and how it functions
- The students can describe the types of PCD and give examples
- The students can contrast the different plant senescence types
- The students know the positive and negative factors influencing the senescence in plants
- The students can distinguish among the various types of whole plant senescence

> Abilities:

- The students can use the obtained knowledge to recognise when a plant starts to senesce
- The students can recognise how senescence influences crop yield
- > Attitude:
- The students see the plant life from a new perspective understanding why plants do not live for ever although they theoretically could
- Autonomy/responsibility
- The students feel responsibility towards old trees
- The students can autonomously argue how to avoid early plant senescence/death

10.1. Plant senescence

Plant senescence is an energy-dependent, autolytic (self-digesting) process that is controlled by the interaction of environmental factors with genetically regulated developmental programs (1).

Levels of Plant Senescence		
PCD (programmed cell death)		
Organ Senescence		
Whole Plant Senescence		

Fig.10.1. Levels of senescence in plants
Senescence can manifest at the level of the whole plant, as in monocarpic senescence, at the organ level, as in leaf senescence, and at the level of cells, e.g. in the tracheary element differentiation (Fig. 10.1.).

We discuss these types of senescence in plants emphasizing the main differences between them and those factors which crucially contribute to their regulations.

10.1.1. Programmed cell death (PCD)

PCD can be induced by specific developmental signals or by potential lethal events, such as pathogen attack (2). PCD is an essential aspect of normal plant development but it can also be induced in response to both biotic and abiotic stress (Fig.10.2.). A characteristic set of genes can express during PCD. Activation of nucleases and proteases can occur in PCD. The most known PCD type in plants is the protection against pathogens. Necrotic lesions can form after infection in order to isolate and prevent from spreading to surrounding healthy tissues. This localized cell death is called hypersensitive response. Hypersensitive response is a genetically programmed process rather than simple necrosis.



Fig.10.2. Programmed cell death occurring in different plant developmental and stress conditions. (Figure of Á. Szepesi)

Hypersensitive cell death, a resistance response to pathogen attack. Condensation and cleavage of DNA in the nucleus precedes vacuole disruption and blebbing of the tonoplast and plasma membranes, the process ends with destruction of organelles, plasma membrane collapse and leakage of the dead cell's contents into the apoplast.

Tracheary element differentiation is a good example of developmental cell death. Swelling and rupture of the vacuole happens as the cell walls undergo secondary thickening and restructuring. Nuclear DNA fragmentation occurs in the later stages, after vacuole collapse. Finally, autolysis eliminates the remaining cytoplasm, leaving an empty cell enclosed by a thickened, reticulated and perforated wall.

Xylem development in woody stem tissues and lysigenous or schizogenous cell death are also good examples for programmed cell death.

10.1.2. Autolysis and autophagy in plants

PCD during normal development occurs via vacuolar swelling and cell rupturing and is called vacuolar-type PCD, whereas PCD during the hypersensitive response occurs via vacuolar water loss and cell shrinkage and is called hypersensitive response-type PCD. Autophagosomes capture damaged cellular constituents and release their contents into the central vacuole to be degraded into reusable monomers. A subset of the autophagy-related genes and proteins regulates the formation of autophagosomes.

The autophagy pathway captures and degrades cellular constituents within lytic compartments. Autophagy is a catabolic mechanism which can protect the cell from the harmful or lethal effects of damaged and unnecessary proteins and organelles. In order to maintain cellular energy levels, autophagy can help the cell to break down or recycle the cellular components, e.g. during starvation. There are two types of autophagy exist in plants, the macroautophagy and the microautophagy (3). In macroautophagy, autophagosomes can form, that is, specialized organelles which enclose the cytoplasmic components and can fuse with vacuole. In the case of microautophagy, tonoplast membrane can invaginate and form small intravacuolar vesicles called autophagic bodies, which are rapidly degraded by lytic enzymes in the vacuole. At the start of autophagy, the ER form a cup-shaped, membranous cisternae called phagophore. Cytoplasmic components destined for degradation such as misfolded proteins, ribosomes, ER, and mitochondria can fuse with phagophore. This phagophore can form a complete autophagosome, which is surrounded by double membranes. The outer membrane can fuse with vacuolar membrane called tonoplast and as an autophagic body can enter the vacuole and be degraded. The monomers from this degradation can reuse as an energy source or building blocks of new structures.

Autophagy-related genes can regulate autophagy (ATG) genes). They are very conserved. The core autophagy machinery controls the initiation and growth of the autophagosome, can contain three main protein groups. ATG9 and its cycling system, including the ATG1/ATG13 kinase complex; the phosphatidylinositol 3-OH kinase (PI3K) complex, and the ubiquitin-like protein system, including ATG12 complex and ATG8. The localization of these groups is the phagophore assembly site of the ER. ATG9 shuttles between the phagophore assembly site and the trans Golgi network and other sites. ATG1 kinase complex is required for this membrane shuttling system, helping the ATG9 to gain new membrane. This transport is important in autophagy, because if this transport is inhibited, autophagy also blocked.

In addition to its role in senescence, the autophagy pathway is a homeostatic mechanism that maintains the metabolic and structural integrity of the cell.

TOR (target of rapamycin) is a serine/threonine protein kinase which is a master switch of controlling the ATG genes. This pathway is crucial metabolic and developmental switch in eukaryotes, by integrating the nutrient and energy signalling to promote cell proliferation and growth. TOR is a negative regulator of autophagy in plants. It can phosphorylate ATG1/ATG13 complex, which cannot bind to the phagophore assembly site. Consequently, ATG9 is not capable to obtain new membrane. Various kinds of stresses can stimulate autophagy by inhibition of TOR pathway.

In plant development, autophagy has a dual role. In non-senescing tissues, autophagy can act as a homeostatic mechanism to maintain the metabolic and structural integrity of the cell. Plants with defective autophagy showed accelerated senescence and reduced root growth compared with control. Autophagy has a negative effect on homeostasis, as well, during the hypersensitive response.

10.1.3. Organ senescence

10.1.3.1. The leaf senescence syndrome

All leaf types undergo senescence, even the leaves of Welwitschia mirabilis, distant relative of pines with its two leaves. The leaf senescence is dependent from age, environmental factors, biotic stress or abiotic stress (5). Leaf senescence uses a specialized type of PCD allowing efficient remobilization of nutrients from the source leaves to the growing, young sink organs across the phloem. Genetically programmed events can occur, e.g. the breakdown of chlorophyll as an early structural change. The chlorophyll sums up to 70 % of the leaf protein (RUBISCO (ribulose-1,5-bisphosphate carboxylase/oxygenase and light-harvesting chlorophyll-binding protein II (LHCPII)). The leaf senescence can contribute to maintain the overall fitness of plant. Hydrolytic enzymes can induce the breakdown and reusing of the cellular proteins, carbohydrates. Minerals also can transport to the sink organs. Leaf senescence can occur under optimal growth conditions, so this is a normal developmental process. Older leaves can be shaded and this situation can induce lower photosynthetic efficiency, triggering senescence of them. Leaf senescence and abscission are coupled programs contributing the optimization of photosynthetic and nutrient efficiency of plants. Leaf senescence involves the breakdown of cellular proteins, carbohydrates, and nucleic acids and the redistribution of their components back into the main body of the plant, to actively growing areas. Minerals are also transported out of senescing leaves back into the plant. The developmental age of a leaf may differ from its chronological age, and it is influenced by internal and external factors.

Leaf senescence may be sequential, seasonal, or stress-induced. There is a senescence gradient from the youngest leaves very close to the tip to the oldest leaves located at the base of shoot, this is called sequential leaf senescence. In the case of seasonal leaf senescence, can occur at once in response to shorter day length and cooler temperatures in the temperate climates, all leaves senescence together. At the level of cells, both types of leaf senescence show vacuolar-type PCD processes. PCD can be manipulated to induce tissues to remain in less mature stages of development.

In unfavourable conditions, leaf senescence can occur prematurely. Soma biotic stresses can induce leaf senescence, e.g. drought, mineral deficiency, high light, UV-B, ozone, darkness. Biotic stresses also can trigger premature senescence.

Morphological features of stress-induced leaf senescence are different from developmental leaf senescence type; as specific site of leaves are affected. Stressed tissue senesces earlier than unstressed tissue.

Developmental leaf senescence consists of three distinct phases. These phases are the initiation phase, the degenerative phase and the terminal phase. In the initiation phase, leaf

can receive some signals (developmental and environmental) initiating a decline in photosynthesis and transition from nitrogen sink to nitrogen source. In the degenerative phase, cellular organelles and macromolecules can undergo autolysis. This phase is responsible for forming abscission layer. In the terminal phase, autolysis is completed and because of cell separation can occur at the abscission layer, leaf abscission is fulfilled.

The chloroplast is the site of the earliest cellular changes during leaf senescence. Catabolism and remobilization of chloroplast proteins add primary sources of amino acids and nitrogen for sink organs. Chlorophyll and its degradation products are dangerous and lethal for cells because of their photoreactive features (5). Chloroplasts can be transformed to gerontoplasts, which resemble to chromoplasts. In the gerontoplasts, grana are unstacked, thylakoid membranes are lost and lipid-like plastoglobuli can accumulate. The efficiency of photochemical reactions and Calvin-Benson cycle is declined. There is a possibility the gerontoplast to transform back chloroplast till the terminal phase of cell death. Nucleus and mitochondria remain intact until the later stages of senescence in order to maintain the gene expression and energy production. Interestingly, the chloroplasts of guard cells are the last organelles to be degraded in the leaf. Typical vacuolar-type PCD symptoms can occur, such as tonoplast breakdown, nuclear condensation, autolysis, starting from the tip to the base.

The autolysis of chloroplast proteins occurs in multiple compartments. Some enzymes are plastid-localized, but other enzymes can be found outside the chloroplasts. Breakdown of rubisco and other proteins can be degraded via two types of autophagic structures, the rubisco-containing bodies and senescence-associated vacuoles. The difference is that rubisco-containing bodies use the autophagy machinery. Rubisco-containing bodies are bordered with a double membrane and can be enclosed by autophagosomes which deliver their contents to the vacuole for further degradation. Senescence-associated vacuoles are small, rich in proteases, acidic vacuoles, which can increase their number in leaf mesophyll and guard cells, but not in the non-green epidermal cells. Senescence-associated vacuoles can directly degrade rubisco by other stromal enzymes but also can fuse with the central vacuole. Autophagy is required for whole breakdown of chloroplast during dark-induced leaf senescence.

The STAY-GREEN (SGR) protein plays an important role in both LHCP II protein recycling and chlorophyll catabolism (4). Chlorophyll is tightly bound in complexes with proteins. During senescence the breakdown of these complexes must be loosened in order to apoproteins be recycled. STAY-GREEN (SGR) is a chloroplast protein which can destabilize the chlorophyll-protein complexes, required for the proteolysis of LHCP II within the chloroplast. Mutants of SGR stay their green colour during senescence because of the uncatabolized chlorophyll contents. Gregor Mendel's peas also show this SGR mutation in the green cotyledon phenotype. However, the decline of photosynthetic efficiency exhibits the same decline like wild type plants. Partial proteolytic cleavage releases LHCP II proteins for autolysis, so chlorophyll molecules can be exported to the cytosol in degraded form and after being stored in the vacuole. Leaf senescence is preceded by a massive reprogramming of gene expression.

Senescence involves the ordered degradation of potentially phototoxic chlorophyll.

Senescence is an end of some cellular events. Some organelles can maintain activity, while others can be inactive.

During the leaf senescence, the chloroplast is the first organelle which can be deteriorated, as the thylakoid and stromal enzymes can be destroyed. This stage requires an active nucleus. Nutrient remobilization is very important, because the 20 % of total cellular nitrogen can come from the chlorophyll binding proteins. However, the destroyed chloroplasts can contribute to the release of phototoxic chlorophylls.

The so called stay-green mutants can help us to understand the chlorophyll degradation pathways in plants. Two types of stay-green mutant plants exist. Functional stay-green mutants retain their photosynthetic capacity and green colour longer than the wild type. Non-functional stay-green mutants are also green but the dismantling of organelles are the same like in the wild type.

10.1.3.2. Leaf senescence and its regulatory network

Gene expression can contribute to the whole senescence processes in plants. In Arabidopsis, 827 genes were identified to increase their expression level during senescence (6). Senescence-associated genes (SAGs) are those genes which are upregulated and can contribute to upregulation of other SAG genes. Senescence can repress gene expression; these are the senescence-down-regulated genes (SDGs). SAGs include many genes which play a role in autophagy, abiotic or biotic stress processes, lipid breakdown or hormonal signalling pathways. The gene expression pattern of stressed-related leaf senescence has differences as compared to developmental leaf senescence but only at the earlier phases.

A network of overlapping signalling pathways integrates and external input to regulate senescence through gene expression.

The leaf senescence is dependent from internal and external factors. Internal factors are plant hormones (e.g. salicylic acid), signalling molecules, or developmental ages of plants (7). External factors involve seasonal changes, stress factors (biotic or abiotic). There are some overlapping signalling pathways which can contribute to the leaf senescence. Leaf senescence is preceded by a massive reprogramming of gene expression. ROS-based signalling, the ubiquitin-proteasome pathway, protein kinases and phosphatases as well as MAP kinases or hormonal signalling. These factors can alter gene expression and activating or repressing transcription factors. Chromatin remodelling and histone modification processes also can contribute to the senescence in leaves. Senescence-associated genes (SAGs) and senescence associated proteins directly promote the process of leaf senescence. The NAC and WRKY gene families are the most abundant transcription factors regulating leaf senescence. NAC and WRKY gene families are highly conserved and are the most abundant transcription factors in the regulation of senescence (6). NAC transcription factors (name after the related NAM, ATAF, and CUC gene families in different species) one of the most abundant families during senescence. These transcription factors can contain a highly conserved N-terminal DNAbinding domain and a variable C-terminal regulatory domain. These are one of the most plant -specific transcription factors, in Arabidopsis they are encoded by 105 genes. In cereals, functional NAC allele induces earlier leaf senescence and nutrient retranslocation. This NAC

allele is not functional in domesticated wheat species. However, domesticated wheat varieties contain two other related NAC genes, which lack the frameshift mutation and therefore they are functional as senescence accelerators. There are some evidences that delayed senescence can result in reduced grain protein and mineral nutrient content, so there is a crucial role of nutrient retranslocation during senescence for normal grain development. WRKY transcription factors are plant-specific group of transcription factors, involved in developmental processes. They have a 60 amino acid region named for the conserved amino acid sequence WRKYGQK in its N-terminal domain. Not just in senescence but also in plant-pathogen interaction they have an important role. Knockout mutant Arabidopsis plants of the WRKY 53 gene showed delayed leaf senescence. The direct targets of WRKY53 can contain the promoter of several SAGs and other WRKY related genes. WRKY22 is involved in the dark-induced senescence of leaves.

There is growing evidence that reactive oxygen species (ROS), especially H2O2, can serve as internal signals to promote senescence. ROS serve as internal signalling agents in leaf senescence. Reactive oxygen species (ROS), especially hydrogen peroxide (H2O2) can act as a signals for activating genetic programs which can regulate cell death events. During leaf senescence, the plant antioxidants decrease while ROS levels are increasing.

WRKY53 can act as a regulator switch controlling the leaf senescence. Its gene expression is increasing when leaves arrive at the bolting period and H2O2 levels are also increasing. Treatment of leaves by H2O2 also induce WRKY53 gene expression, so it means that H2O2 can induce leaf senescence in Arabidopsis.

Sugars also induce leaf senescence as signalling molecules. High sugar concentrations lower photosynthetic activities and trigger leaf senescence. High concentrations of sugars may also serve to signal leaf senescence, especially under conditions of low nitrogen availability.

Plant hormones can overlap and interact in the regulation of leaf senescence

Leaf senescence is governed by developmental or chronological age, but the timing and progression of the process are flexible. This flexibility can be adjusted by hormones, which can accelerate or repress the senescence processes (Fig.10.3.). Leaves must reach a maturation stage to respond or be competent to senescence. Plant hormones interact to regulate leaf senescence, though they are only effective at promoting senescence once the leaf reaches a certain stage of maturity.

Positive senescence regulators are ethylene, abscisic acid (ABA), jasmonic acid (JA), and brassinosteroids and salicylic acid. Ethylene is a hormone which can play a role in development and growth as well as in senescence. Ethylene treatment can cause shedding of leaves and flowers, while inhibitors of ethylene synthesis can delay senescence. Ethylene-insensitive mutants can show delayed senescence phenotype in Arabidopsis thaliana. Ethylene is not enough to onset and progression of senescence, maybe ethylene signalling regulates the later stages of leaf senescence, since ethylene synthesis related transcripts can appear around the time when chlorophyll begin to degrade.

Abscisic acid (ABA) can increase in senescing leaves promoting senescence syndrome and expression of several SAGs. Like ethylene, ABA is not a triggering factor of senescence but also enhancer of it. ABA synthesis and signal pathway is upregulated and consequently the ABA level is increasing. Environmental stress factors also induce ABA synthesis and induce leaf senescence. ABA or salt stress can induce the NAC transcription factor VNI2 (VND-INTERACTING 2) upregulation during leaf senescence. ABA-induced stress signalling and leaf senescence signalling are overlapping in leaves. Senescing leaves contain ABA induced SAG113, which is a gene encoding protein phosphatase 2C, a negative regulator of ABA signal pathway. ABA can induce SAG113 transcription, which inhibit the ABA-induced stomatal closure resulting in increased water loss and accelerated senescence.



Fig.10.3. Hormonal influences on leaf senescence and chlorophyll degradation processes in plants. SAGs, senescence-associated genes; SAUR, small auxin up RNA gene; SARK, senescence-associated receptor-like kinase; SAUL, senescence-associated E3 ubiquitin ligase. Arrows indicate senescence promoting pathways. Lines terminated by bars indicate inhibitory pathways. Dotted lines indicate the mechanisms may be indirect. For details on the genetic components associated with these pathways, see Fischer (2012) and Khan et al. (2013) (Figure of A. Fehér; adapted from Griffiths et al, 2014).

Jasmonic acid (JA) JA stimulates leaf senescence as positive regulator controlling the expression of a series of senescence-related genes. In the *coi* mutant plants (COI is a JA receptor in Arabidopsis), did not show accelerated senescence. Jasmonate concentration increase by leaf age. However, *coi* mutant plants showed floral abscission delaying, suggesting the role of JA in floral senescence. The senescence accelerating effect of JA is age-dependent. Older leaves respond more rapidly than young leaves.

Brassinosteroids (BRs) are positive regulators of leaf senescence. The application of brassinosteroids accelerates senescence while BR-deficient mutant plants show delayed

senescence. The delayed senescence of BR mutants is also associated with phenotypic alterations, indicating that this process is a secondary effect of the altered development. Results suggest that BRs can play a role as a global regulator in leaf development, rather than a regulator of leaf senescence.

Salicylic acid (SA) also accelerate leaf senescence. SA contents of leaves can increase by the age and also at the time when chlorophyll can start to degrade. About 20 % of SAGs are upregulated by SA signalling pathway. SA treatments also induce expression of many SAGs e.g. WRKY53, which is a master switch regulator of senescence.

Negative senescence regulators: senescence-repressing regulators include cytokinins, auxins, and gibberellins.

Cytokinins can repress the senescence in all plants. The effect is direct even if it is used as local treatment or leaf spraying. Green islands can appear on the leaves with high cytokinin contents.

Mature leaves contain less cytokinin, and depend from the root-derived cytokinins to postpone the senescence. Cytokinins are natural regulators of leaf senescence. The AHK3 receptor can contribute to the delayed senescence. Molecular mechanisms remain unclear behind the cytokinin action delaying senescence. Cytokinins can regulate nutrient remobilization and alterations of source-sink relations. Nutrients can move toward the cytokinin-treated tissues. However, cytokinins can also induce the mobilization of non-metabolizable substrate analogues as well.

Auxin has a complex role in plant development and leaf senescence. High auxin concentrations can induce ethylene biosynthesis, which can promote the senescence of old leaves. Exogenous auxin treatments can delay the senescence decreasing the expression of many SAGs. YUCCA6 catalyses the rate-limiting step of auxin biosynthesis, can delay the senescence decreasing SAG expression.

Gibberellins are senescence repressing hormones in active forms. Active forms can decline in leaves with the age. Biologically active GA is removed during leaf senescence. Leaf senescence is inhibited by biologically active form of GAs.

10.1.3.3. Leaf abscission

Abscission can occur when leaves, fruits, flowers or other organs can shed from the plant. This can be localized in a special zone called abscission zone, which is located near the base of the petiole and can contain specific layers of cells. This abscission zone can differ many months before the organ separation. Prior to abscission, a separation layer forms within the abscission zone. Cell walls are dissolute in these cells without any cell death and results in the leaf shedding. The exact time of leaf abscission is regulated by the interaction of ethylene and auxin. Ethylene has an important role in the activation of the events leading to cell separation within the abscission zone. The process of leaf abscission can be divided into three developmental phases, in order to become competent to respond to ethylene. The first is the leaf maintenance phase. The leaf is healthy and fully functional. The abscission zone is maintained by a gradient of auxin from the leaf blade to the stem. The second phase is the

abscission induction phase. Auxin gradient is reduced from the leaf blade, which is normal in the case of leaf senescence, and causes the abscission zone to become more sensitive to ethylene. Inhibitors of auxin synthesis or transport in the leaf can result in enhanced leaf senescence promoting the abscission.

The final phase is the abscission phase. In the abscission zone, cells are sensitized and respond to low concentrations of endogenous ethylene by synthesizing and secreting cell wall degradation enzymes and cell wall remodelling proteins, e.g. cellulases, polygalacturonases, hydrolases, and expansin, resulting in cell separation and leaf abscission.

Abscission initiation regulator genes were discovered by mutations inhibiting flower abscission in Arabidopsis. IDA (INFOLRESCENCE DEFICIENT IN ABSCISSION) is a small secreted peptide and its probable receptors, leucine-rich repeat receptor-like kinases HAESA (HAE) and HAESA-LIKE2 (HSL2). Binding to IDA, HAE/HSL2 receptor complex is thought to trigger the MAPK kinase cascade, which can lead to the transcriptional activation of genes encoding cell wall loosening enzymes, cell expansion and cell separation.

High levels of auxin keep leaf tissue in an ethylene-insensitive state, but as auxin levels drop, the abscission-promoting and auxin-repressing effects of ethylene become stronger.

10.1.4. Whole plant senescence

In general, annuals and biennials reproduce only once before senescing, while perennials can reproduce multiple times before senescing.

According to some models, whole plant senescence represents an accelerated form of aging where tissues are programmed to fail quickly once certain thresholds are reached.

Nutrient or hormonal redistribution from vegetative structures to reproductive sinks may trigger whole plant senescence in monocarpic plants. While growth efficiency in trees declines with increasing tree size, leaf mass increases as the square of trunk diameter and can overcome this loss in efficiency, until internal or external factors initiate whole tree senescence.

Programmed deaths of plant cells and organs can be benefit for the whole plant fitness in evolutionary term. The life spans of plants vary widely across plant species. Plant life cycles can be annual, biennial or perennial. Some desert plants life span is just about few weeks, while some bristlecone pines can live about 4,500 years. Annual plants can live just for one year, in this period they can grow, reproduce and die at the end of the year. In the case of biennial plants, they grow vegetatively in the first year and the second year is for the reproduction and senescence and death. Monocarpic plants are the annual and biennial plants, because these plants can reproduce just once in their lives. Plants which can live for 3 years or longer and may be herbaceous or woody can be called as perennial plants. These perennial plants are usually polycarpic, producing fruits and seeds over multiple seasons, except the century plant (*Agave americana*) or Japanese timber bamboo (*Phyllostachys bambusoides*).

Many perennial plants that form clones by asexual reproduction. They can proliferate into community-sized individuals that are interconnected and achieve astounding ages, such as King's lomatia (*Lomatia tasmanica*), a Tasmanian shrub in the Proteaceae family that can be over 43,000 years old. Each individual lomatia can live just up to 300 years, but because it does not transfer any senescence signal to its clones, the clonal community apparently grows and proliferates indefinitely.

Whole plant senescence differs from aging in animals. Whole plant senescence of monocarpic plants is simply an accelerated form of aging. Some models suggest that the abilities of long-lived perennials to maintain the integrity of their meristems for thousands of years comes from developmental programs that successfully ward off the deteriorative effects of time.

One type of cellular damage dependent from time is the mutational load. The mutation rate might even increase over time due to the build-up reactive oxygen species (ROS). Some results show that pollen viability can decrease by mutational load. There are some somatic mutations which are age-dependent. However, plants seem to have a high tolerance for genetic mosaicism.

Telomere shortening is another type of age-dependent damage. Telomeres are the regions of repetitive DNA that form the chromosome ends and protect them from degradation. Normal chromosome replication results in telomere shortening. Telomerase is a ribonucleoprotein enzyme complex extends the ends of telomeres after replication through the activity of telomerase reverse transcriptase. Interestingly, Arabidopsis mutant plants lacking telomerase activity grow and reproduce for up to 10 generations.

The determinacy of shoot apical meristems is developmentally regulated

Plants have indeterminate growth due the activities of the apical meristems, but the determinacy of the apical meristems is under strict developmental control. The growth habits, life cycles, and senescence profiles of different plant species are intimately connected to their patterns of apical meristem determinacy.

Monocarpic plant species have indeterminate vegetative apices which can become to determinate floral apices, and the entire plant can senescence and dies after seed dispersal. However, polycarpic perennials retain a population of indeterminate shoot apices and apices which can become reproductive and determinate. Monocarpic senescence typically contains three coordinated events, senescence of somatic organs and tissues such as leaves, the growth arrest and senescence of shoot apical meristems, and suppression of axillary buds (8).

Axillary bud suppression during monocarpic senescence has been investigated in Arabidopsis thaliana plants. AtMYB2 is a transcription factor which expressed in basal internode suppressing both the cytokinin biosynthesis and branch formation during monocarpic senescence. Those T-DNA mutants which lacking functional AtMYB2 protein are bushy because of the increased cytokinin production, which indicates the importance of cytokinins as negative regulators of whole plant senescence in plants.

Nutrient or hormonal redistribution may trigger senescence in monocarpic plants (8).

It is well known that removal of the reproductive structures can help to delay the senescence. It is based on the fact that nutrients redistribute via the phloem from vegetative sources to reproductive sinks. Alterations of source-sink relations can induce senescence. Cytokinins increase sink strength in leaves and also delay leaf senescence. In peas, high endogenous GA levels in the vegetative buds can induce high sink strength, vigorous vegetative growth and delayed whole plant senescence. High auxin levels in floral buds are correlated with high sink strength of the reproductive structures and rapid reproductive development followed by whole plant senescence. Some results show that male or female plants of dioecoius plants can be triggered senescence the same ratio. The nutritional demands of stamine flowers exceeds the nutritional demand of pistillate flowers, thus could be a regulating factor for monocarpic plant senescence.

Critical compound is not the carbohydrate, but exogenous sugars can trigger leaf senescence. Alterations in source-sink relations can cause an increase in the ratio of C:N, which is associated with the vacuolar-type PCD in senescing leaves.

The rate of carbon accumulation in trees increases continuously with tree size

The growth rate of trees declines with increasing tree size and mass. Leaf photosynthetic efficiency declines with increasing age of the tree. This process is inevitable consequence of increasing resource allocation to reproduction. There are some parameters which can reflect properly these age-related declines: net primary productivity and the rate of mass gain per unit leaf area.

Although growth efficiency often declines with increasing tree size, total tree leaf mass increases as the square of trunk diameter.

Whole tree senescence is caused by massive organ failure over a relatively short time, rather than by a slow decline due to aging. Beside internal factors, external factors also can induce whole plant senescence, such as fire, nutrient depletion, water stress or pathogen attack, as well as herbicide treatments, but these are very poorly understood mechanisms.

Plant cells, organs, and organisms experience wear from the effects of aging and from external stress. To break down old or damaged tissues, or to further some developmental pathways, plants undergo senescence, or genetically programmed cell death. Senescence differs from necrosis, which is the unexpected death of tissues caused by physical or chemical damage or other external agents.

Summary

- 1. Leaf senescence is adaptive and strictly regulated.
- 2. Plants exhibit various types of senescence.
- 3. Monocarpic senescence is the type when the entire plant goes across senescence after a single reproductive cycle.
- 4. In deciduous trees, there is a seasonal leaf senescence.

- 5. Sequential leaf senescence occurs if leaves can reach a certain age.
- 6. External and internal factors can induce senescence processes.
- 7. Leaf senescence strongly depends on the metabolic status and oxidative homeostasis.
- 8. Senescence regulates an ordered degradation of phytotoxic chlorophyll.
- 9. Programmed cell death is a specialized type of senescence.

Review Questions

- 1. What is the difference between senescence and necrosis in plants?
- 2. Which type of senescence can occur in plants?
- 3. What is the leaf senescence syndrome?
- 4. Which factors can induce of senescence in plants?
- 5. Which hormones can be involved in senescence processes as positive regulators?
- 6. What are the negative regulators of senescence?
- 7. Which processes can induce the whole plant senescence?

Discussion Questions

- 1. What is the main differences between senescence types in plants?
- 2. How can the features of programmed cell death be detected in plants?
- 3. Why is the effect of auxin so complex in senescence processes?
- 4. What is the role of cytokinins in the senescence processes?
- 5. Is there any plant which can live for thousand years?

Additional Reading

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