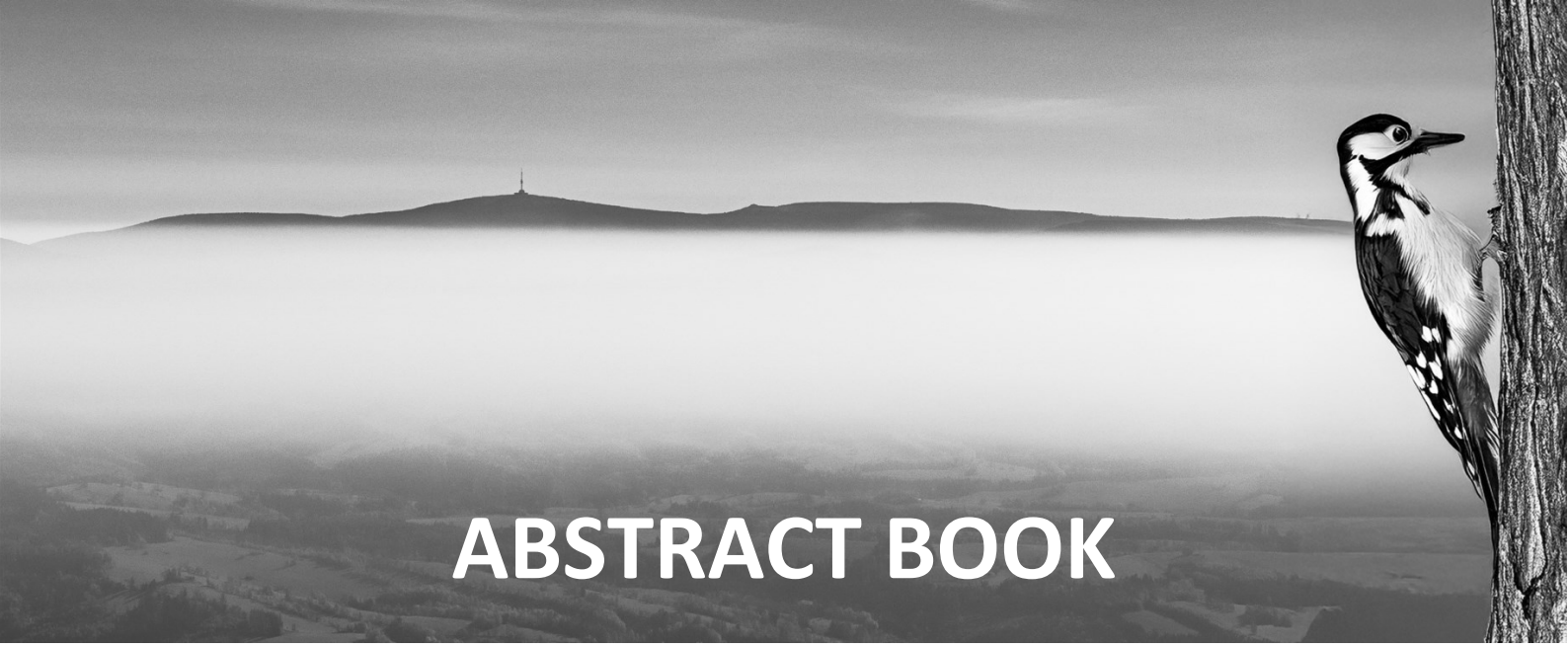

ABSTRACT BOOK

PHYTOHORMONES AND RELATED SUBSTANCES, BIOSTIMULANTS AND PLANT BIOTECHNOLOGIES

2025

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MINISTRY OF EDUCATION, YOUTH AND SPORTS

GH3-mediated auxin modulation fine-tunes seed germination through hormonal cross-Talk

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The transition from dormancy to germination is a tightly regulated developmental process controlled by multiple phytohormones. Abscisic acid (ABA) and gibberellins (GA) serve as key antagonistic regulators, with ABA reinforcing dormancy and GA promoting germination. While auxin (indole-3-acetic acid, IAA) is well known for its roles in plant growth and development, its contribution to seed germination remains limited. Emerging evidence suggests that auxin interacts with ABA signalling to maintain dormancy, potentially delaying germination[1,2]. However, it remains unclear whether precise regulation of endogenous IAA levels influences germination kinetics and dormancy release.
Therefore, we characterized the temporal and spatial expression of Arabidopsis IAA-conjugating Subgroup II GH3 genes during germination, revealing stage-specific expression patterns linked to radicle emergence and seedling establishment. Surprisingly, lines overexpressing GH3.3, GH3.4, and GH3.17 (exhibiting reduced endogenous IAA) and the gh3 sextuple mutant (with elevated endogenous IAA) displayed accelerated germination and decreased sensitivity to exogenously applied auxin. Chemical inhibition of GH3 enzymes with kakeimide further confirmed that IAA conjugation is essential for optimal germination kinetics. We previously demonstrated GH3.3 localization in cytosol and nucleus[3] and now extend this analysis to GH3.4 and GH3.17 under native promoter conditions. Moreover, dexamethasone-inducible lines revealed that GH3-dependent germination phenotypes arise exclusively from their enzymatic activity, independent of their subcellular localization. Hormonal supplementation assays uncovered a dual auxin-mediated regulatory framework: elevated endogenous IAA promoted germination via ABA-dependent pathways, whereas reduced endogenous IAA enhanced germination through GA-dependent pathways.
Collectively, our findings establish GH3-mediated auxin homeostasis as a modulator of seed germination. By fine-tuning IAA levels, GH3 enzymes integrate auxin signalling with ABA-GA crosstalk, thereby balancing dormancy release and germination progression. These insights deepen our understanding of hormonal regulation in seed biology and open new avenues for optimizing germination strategies.

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3H-pyrazolo[4,3-f]quinoline-based compounds as novel kinase inhibitors against FLT3-driven leukemia

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Acute myeloid leukemia (AML) is a severe blood cell disorder characterized by rapid progression that can lead to death within weeks to months if left untreated.
In a large number of patients, altered FLT3 activity has been observed. In approximately one-third of patients, a mutation in the gene itself has been detected, leading to its continuous activation and resistance to certain established treatment methods. The two most important mutations include FLT3-ITD and FLT3-TKD, highlighting the need to develop drugs capable of targeting these specific FLT3 mutations.
One class of potential therapeutics includes compounds based on 3H-pyrazolo[4,3-f]quinoline, which have already demonstrated the ability to inhibit AML cell growth at nanomolar concentrations. Some of these compounds have also shown the capacity to specifically target the oncogenic FLT3-ITD mutation and block its phosphorylation.
The authors would like to acknowledge the support of the grants R01-CA267978 (NIH) and LUAUS24174 (program INTER-EXCELLENCE II) from the Ministry of Education, Youth and Sports of the Czech Republic.

Regulation of ALDH6 enzymes across species: structural insights and effects of protein interaction

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The aldehyde dehydrogenase (ALDH) superfamily includes $NAD(P)^+$ dependent oxidoreductases providing oxidation of aldehydes to the corresponding carboxylic acids. Few members (e.g., ALDH6 or ALDH18) also exhibit additional catalytic functions. However, these functions remain poorly characterized.
The methylmalonic semialdehyde dehydrogenase (ALDH6; EC 1.2.1.27), is a NAD^+ and CoA dependent member involved in the catabolism of branched amino acids and uridine. ALDH6 contributes to anaplerotic reactions feeding the TCA cycle with acetyl-CoA or succinyl-CoA. Despite its metabolic importance, details as CoA binding, oligomerization dynamics, or substrate specificity remain unclear. Comparisons across species are also limited as the only structure of bacterial and human isoforms were resolved.
This study presents a comparative analysis of ALDH6 isoforms from barley (*Hordeum vulgare*), moss (*Physcomitrium patens*), and human (*Homo sapiens*). Key residues involved in substrate recognition were identified and investigated via site-directed mutagenesis. Crystal structures of HvALDH6 and PpALDH6 revealed modifications in usually highly conserved NAD^+-binding domain that may enable CoA binding. Additionally, a novel tetramer-tetramer interaction mediated by a C-terminal nonapeptide was discovered, involving an active-site arginine conformation changes that affects both enzyme activity and substrate affinity for malonic and methylmalonic semialdehyde.

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Optimization of a direct injection method for the determination of auxins in plant material using HPLC-MS

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Auxins represent a group of phytohormones involved in the regulation of plant growth and developmental processes. They influence cell growth and differentiation, tropic responses, and overall plant morphogenesis. Their effects are highly dependent on concentration, localization, and the developmental stage of the tissue.
The analytical determination of auxins is challenging due to their low concentrations and the complex nature of plant matrices. This places high demands on the sensitivity of the analytical method. Standard approaches typically involve complex protocols that include sample purification, most commonly using solid-phase extraction (SPE), to reduce matrix content and minimize matrix effects. While this step is justified, it also has drawbacks, such as potential analyte loss, time consumption, and the risk of losing volatile compounds during evaporation.
The direct injection method is based on the application of an extract without prior purification. The advantage is the elimination of steps where analyte losses might occur and a simplification of the overall workflow. On the other hand, this increases the demands on the performance and robustness of the analytical instrument, especially due to matrix effects. Such methods are commonly used in human metabolomics, where matrices (e.g., plasma) are significantly simpler than plant samples. Therefore, we decided to explore the possibilities of using this strategy for the analysis of phytohormones.
It has been shown that 10% methanol is a suitable solvent for auxin extraction and is also compatible with established chromatographic methods. This eliminates the need for solvent switching and allows extraction and analysis in the same environment. However, the overall signal in mass spectrometry can be influenced not only by the choice of solvent but also by the extraction volume, sample weight, and injection volume. The goal of this study is thus to identify the most suitable combination of these three parameters. Since the final values are influenced by matrix effects and extraction capacity, a stepwise change of individual parameters does not always reliably lead to the true optimum. Therefore, we adopted a factorial experimental design approach, which allows simultaneous optimization of all parameters using multiple linear regression.
Preliminary results suggest that with thorough optimization, the direct injection method can be effectively used in plant metabolomics and phytohormone analysis.

This work was supported by the ERC Synergy project STARMORPH (No. 101166880) under the European Union's Horizon Europe research and innovation program.

Interaction of light and ABA signaling in plant responses to salt stress

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In recent years, research focused on plant stress is becoming more and more important due to deteriorating environmental conditions. Salt stress has emerged as a major agricultural problem, limiting plant growth and development. It is a primary factor causing soil degradation, leading to reduced soil fertility and crop productivity. Understanding the interplay between light and the abscisic acid (ABA) signaling pathway is pivotal in uncovering how plants adapt to environmental stress conditions. ABA is a key hormone in plant response to stress and its level is altered by light conditions. This fact indicates that light can play a significant role in plant adaptation to stress.
This study focuses on a tomato (*Solanum lycopersicum L.*) as a model organism to investigate the mechanisms behind plant responses to salt stress. Specificly *hp1* (*high pigment 1*) mutant, characterized by a defect in the *DDB1* (*UV-DAMAGED DNA BINDING 1*) protein, which is involved in photomorphogenesis, stress responses and stability in plants.
Experiments with 7-day-old seedlings revealed that *DDB1* deficiency in *hp1* mutant enhances light sensitivity through intensified light signaling. This altered light signaling promotes higher expression of the *HY5* gene in

mutant plants. *HY5* is a key transcription factor in the regulation of light-mediated responses. Further mutation in *DDB1* protein significantly affected ABA biosynthesis and signaling, particularly influencing the expression of the *ABI5* gene, which acts as a convergence point between the light and ABA pathways. These findings suggest that light and ABA closely cooperate in the plant's response to stress.
The results of this study provide insight into the mechanisms linking light and ABA signaling pathway during salt stress. This knowledge can contribute to a better understanding of how plants respond to stress condtions and may have potential applications in improving stress tolerance in crops.

Study of salicylic acid metabolism in plants using mass spectrometry

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Plant immunity is defined as the capacity of plants to prevent or ward off biological attacks by pathogens. It involves the recognition of the pathogen by specific receptors and the triggering of signalling pathways leading to processes that help plants to defend themselves. The defence signalling is mediated by cross-communication of groups of plant hormones. Salicylic acid (SA) is one of the most pronounced plant hormones involved in control of immunity and defence mechanisms in plants. Defence related SA accumulation comes from its biosynthesis, transport and possible release from some of its metabolites. The involvement of SA biosynthetic pathways as well as extend of SA metabolism in various plant species are still under investigation. Liquid chromatography tandem mass spectrometry (LC-MS/MS) is one of the most commonly used analytical technics in plant hormone quantification. The selectivity and high sensitivity enables to track typically low concentrations of phytohormones. The comprehensive multiclass phytohormone LC-MS/MS profiling methods usually include only SA (as the active compound) from the group of SAs. LC-MS/MS methods for determination of defence related phytohormones focus on SA, or some on its biosynthetic precursors or metabolites. In this study, we focus on identification of new SA metabolites and development of liquid chromatography mass spectrometry methods.

Polyamine and ethylene biosynthesis dynamics in *Arabidopsis thaliana* and *Solanum lycopersicum*

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Polyamines are ubiquitously present in all living organisms. In plants, together with phytohormone ethylene, their metabolism plays a crucial role in plant stress and ontogenesis. We have evaluated differences in the responses of model plants *Arabidopsis thaliana* and *Solanum lycopersicum* to abiotic stresses and metabolic modulators based on key metabolite levels. Previous approaches have often focused separately on either polyamines, amino acids, or ethylene precursors. As these pathways are directly interconnected, their simultaneous evaluation can significantly impact our understanding of their core mechanisms.
We have therefore developed a novel and validated liquid chromatography-tandem mass spectrometry based method, enabling quantification of fourteen compounds: the amino acids L-arginine, L-citrulline, L-ornithine; biogenic amines N^{α}-acetyl-L-ornithine and agmatine; the polyamines putrescine, spermidine, spermine,

thermospermine, *N*-acetylputrescine, cadaverine, homospermidine; together with methionine and 1-aminocyclopropane-1-carboxylic acid, serving as key non-volatile precursors of ethylene.
Our analysis revealed distinct metabolic responses between *Arabidopsis* and tomato, highlighted by species-specific differences in polyamine metabolism and ethylene precursors dynamics. Drought and salinity stresses triggered fundamentally different metabolic adjustments, with drought consistently inducing higher metabolite levels and spermine showing stress-specific responses. Metabolic inhibitor treatments with aminoguanidine and L-norvaline, targeting polyamine catabolism and biosynthesis, respectively, revealed further divergencies, mainly demonstrated as significant variations in ethylene precursor levels. Additional experiments with *Arabidopsis* mutants affected in ethylene synthesis and arginine metabolism pathways, as well as methionine treatment, further confirmed the interconnected nature of these metabolic networks and their responses to pathway perturbations. For all approaches, *Arabidopsis* displayed more pronounced metabolic fluctuations compared to tomato. These results provide direct insights into contrasting metabolic plasticity and the interconnected roles of polyamines, amino acids, and ethylene precursors in plant responses and adaptations.

Presented work was supported by The Czech Science Foundation (GAČR) via 20-25948Y junior grant, by Internal Grant Agency of Palacký University (IGA_PrF_2025_019) and by the "Biorefining and circular economy for sustainability" (TN02000044) grant.

Effect of stress on ethylene and other phytohormone levels in Arabidopsis

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Ethylene, a key gaseous phytohormone, regulates plant growth, senescence, and adaptive responses to abiotic stresses like drought, salinity, and extreme temperatures.
By modulating gene expression and signaling pathways, ethylene enhances plant resilience and adaptability, thereby influencing overall crop productivity[1]. A comprehensive understanding of ethylene's mechanisms in stress response is imperative for advancing agricultural practices aimed at mitigating the impacts of climate change on crop yield and stability. Exploring these mechanisms offers promising avenues for developing stress-tolerant plant varieties, essential for ensuring food security in an increasingly volatile environment[2].
This study investigates the impact of altered ethylene production in Arabidopsis mutants (ethylene overproducer *eto2* and ethylene-limited biosynthesis *aco2aco3* mutants) on the profiles of various phytohormones under abiotic stress conditions, specifically heat shock, salinity stress, and their combination. We employed a validated method to accurately measure levels of 1-aminocyclopropane-1-carboxylic acid (ACC), the ethylene precursor, along with other phytohormones. This approach allowed us to assess the interactions between ethylene and other hormonal signals under stress conditions. Our study demonstrates significant interactions among the ACC, abscisic acid (ABA), salicylic acid (SA), cytokinin, and other phytohormones. Stress-induced alterations in ACC levels enhanced the heat shock response in both Col-0 and the aco2aco3 mutant; however, this effect was not observed in the eto2 mutant, and showed similar increases under salinity stress and combined salinity and heat shock conditions across Col-0, *aco2aco3,* and *eto2* seedlings.

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Effects of smoke-water, seaweed extract, vermicompost leachate, and their synergistic interaction on maize germination

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Modern agriculture's reliance on synthetic agrochemicals has raised concerns over environmental sustainability, prompting exploration of eco-friendly alternatives such as natural biostimulants. This study evaluates the individual and synergistic effects of three biostimulants, smoke-water, seaweed extract (Kelpak®), and vermicompost leachate on maize seed germination, seedling growth, enzymatic activity, and phytohormonal profiles under light and dark conditions. Maize seeds were treated with various concentrations of each biostimulant and a combined formulation, and germination performance, α-amylase activity, and phytohormone levels were assessed. Results showed that vermicompost leachate and the biostimulant treatment significantly enhanced root length and seedling vigour index, particularly under dark conditions. Combined biostimulant treatment also elevated α-amylase activity under both light and dark regimes, indicating improved metabolic activation during germination. Phytohormone analysis revealed that BS reduced levels of auxin, its conjugates, and abscisic acid under light, suggesting biostimulant-mediated hormonal modulation. No significant hormonal changes were observed under dark conditions, except for decreased oxIAA in BS-treated seedlings. These findings demonstrate that biostimulant combinations can exert synergistic effects, enhancing early growth and metabolic activity while modulating hormone levels in maize seedlings. The study highlights the potential of integrated biostimulant strategies to support sustainable crop production.

Development of a new generation of plant growth retardants

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In modern agriculture, fertilizers, pesticides, and herbicides are essential for crop growth, but their use often leads to environmental harm, prompting the search for natural, non-toxic alternatives. Gibberellins (GAs) are key plant bioregulators that play a central role in controlling plant growth, making them prominent natural alternatives to synthetic counterparts.[1,2] The work of Prof. Mander, who successfully developed the first "anti-gibberellin structure" in the late 20th century, demonstrated the potential of this approach. These compounds have shown significant effectiveness as competitive inhibitors of natural gibberellins, showing promising results even in limited field trials.
However, high production costs, limited applicability, and the need for large application quantities have significantly constrained their widespread use. To address these challenges, we developed strategies to (1) enhance bioavailability and (2) improve water solubility of these compounds. To overcome the first issue, a

fluorinated derivative of the lead structure was synthesized, aiming to improve the migration of the target molecule through the cytoplasmic membrane. To achieve the second goal, water-soluble ammonium and potassium salts of anti-gibberellins were prepared, facilitating easier application and significantly increasing crop yields in field trials compared to the free carboxylic acid.
However, recent analyses of plant extracts have revealed that anti-gibberellins are rapidly oxidized within plants, producing a metabolite that not only loses its inhibitory activity but unexpectedly promotes growth. The structure of this metabolite was proposed, synthesized, and confirmed in plants, prompting the development of new oxidation-resistant anti-gibberellins. These compounds offer the potential for increased stability, efficiency, and sustainability, offering a promising solution for environmentally friendly agriculture.
In this contribution, we present the latest results obtained during this project.

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Equilibrative Nucleoside Transporter 1 drives cytokinin riboside uptake in Arabidopsis roots

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Cytokinins (CK), essential plant hormones derived from adenine, are classified into active forms (CK free bases), transport forms (CK ribosides), and storage forms (CK glucosides) based on their function and activity. CK ribosides are more resistant to metabolic changes compared to their active forms, which allows them to be transported over both short and long distances within plants. In Arabidopsis, members of the Equilibrative Nucleoside Transporter (ENT) family are specifically responsible for transporting CK riboside forms.
Here, we introduce Equilibrative Nucleoside Transporter 1 (ENT1), which not only actively participates in CK riboside transport but also stands as the primary candidate for CK uptake by roots. Our GUS-promoter analysis also confirmed high expression in the epidermis of the root apical meristem.
We generated a novel *pENT1::ENT1-T53-eGFP-K54-3'-UTR* line, which demonstrated dual localization on both the plasma membrane and tonoplast.
Upon expressing the *pENT1::ENT1-T53-eGFP-K54-3'-UTR* construct in the Col-0 background, this line exhibited higher and specific sensitivity towards CK ribosides. Notably, even more pronounced sensitivity to CK ribosides was demonstrated utilizing the inducible *XVE::ENT1* lines in *TCSv2::NLS-3XVenus* background.
The robust and specific expression pattern of *ENT1* in the root epidermis suggests its role as a gateway for CK from the surrounding environment.

Cytokinin down-regulates photosystem II photochemistry during prolonged darkness in a phytochrome B-dependent manner

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Cytokinins are known to delay dark-induced leaf senescence by preventing the breakdown of photosynthetic pigments - particularly chlorophyll - and proteins, thereby sustaining photosynthetic activity. However, the precise molecular mechanisms through which cytokinins influence photosynthesis are not fully understood. In this study, we examined the effects of the classical aromatic cytokinin BAP and the cytokinin-derived compound MTU on detached Arabidopsis leaves during different stages of dark incubation. Interestingly, although both compounds delayed senescence, they initially suppressed photosystem II (PSII) photochemical activity by enhancing non-photochemical energy dissipation. Despite this early decline, cytokinin-treated leaves preserved PSII function and significantly slowed chlorophyll loss at later stages. Transcriptomic analyses indicated that many cytokinin-responsive genes in dark-incubated leaves are linked to PSII activity and red-light signaling. Additional experiments revealed that this early suppression of PSII photochemistry is dependent on phytochrome B (phyB) and can be reversed by far-red light, suggesting that cytokinins help maintain active phyB even in darkness. We propose that the phyB-dependent modulation of PSII by cytokinins serves as a protective strategy, minimizing photo-oxidative stress when leaves are re-exposed to light after prolonged darkness.

From SH-SY5Y to stem cells: towards better screening of neuroprotectants

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Neurodegenerative diseases (ND) encompass a diverse group of conditions characterized by the progressive, gradual loss of neurons, resulting in severe cognitive and physical impairments. Among the most prominent are Parkinson's and Alzheimer's diseases, both of which are increasing in prevalence as the global population ages. In 2009, approximately 50 million people were affected by ND worldwide[1]. Current projections estimate that over 115 million individuals will be affected by 2050. The development of ND is a complex and multifactorial process, involving genetic predispositions, environmental exposures, aging, and cellular dysfunction. A major

challenge in managing these diseases is the lack of curative treatments; current therapies focus only on symptom management and improving quality of life.

Our research focuses on identifying new compounds with neuroprotective potential. In order to evaluate these effects, we utilized the SH-SY5Y human neuroblastoma cell line, differentiated with 10 µM all-trans retinoic acid to induce neuron-like characteristics. Although this model offers practical advantages such as ease of use and reproducibility, its cancer-derived origin limits its physiological relevance, particularly in mimicking the complex environment of human neurons. To overcome these limitations, we are currently developing a more representative *in vitro* model using neuronal stem cells. This system offers a closer approximation to human neurobiology and pathophysiology, especially when combined with disease-relevant toxins and stress conditions, allowing for more accurate assessment of compound activity.

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Technologically efficient approaches to the preparation of cytokinin conjugates with anti-senescence effects and potential application in human skin models

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The aim of this presentation is the synthesis and determination of biological activity of the new cytokinin derivatives with potential antisenescence and antibacterial and antifungal effect against biotic stress caused by (Pseudomonas syringae, Botrytis cinerea, *Heterodera schachtii*). Theoretical part includes general information about cytokinins, their classification and practical utilization. Separated sections characterize antisenescence and anti-aging activity of cytokinins. The following sections are focused on synthesis and the characterization of new cytokinin derivatives and overall summary of the results of diploma thesis. In the experimental part biological and chemical methods are listed and specific methods of chemical synthesis for the preparation of 6-substituted purine-9-β-D-mannopyranosides are approximated and characterized.

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In Pursuit of Cytotoxic Triterpenoids: Functionalization of Lupane, Taraxastane, Friedelane, and Baccharane Derivatives via Oxidation with Selenium Reagents

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A series of triterpenoids of the lupane, taraxastane, friedelane and baccharane type were oxidized using selenium dioxide (SeO_2) and benzeneseleninic anhydride (BSA) under various conditions. Depending on the reaction conditions, different reaction pathways were observed, including dehydrogenation, allylic oxidation, and 1,2-diketone formation. In this way, derivatives functionalized in the triterpene core (especially in rings A, D, and E), difficult to obtain by other methods, can be easily prepared. The key reaction pathways were investigated using density functional theory (DFT), focusing on bond length variations and transition states, revealing energetically favored pathways and critical transition structures, including covalent and noncovalent interactions. Cytotoxic activity of selected derivatives was investigated. Derivatives **4** and **38** showed strongest cytotoxicity in cancer cells and fibroblasts (IC_{50} 2.6 - 26.4 µM); some compounds were selective for G-361 or HeLa cells. These results suggest that they may find application in pharmaceuticals.
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From organelle to organelle – plant hormone distribution at the subcellular level

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Plant hormones, also known as phytohormones, are naturally occurring organic compounds that act as bioactive signaling molecules and significantly impact the physiological processes and development of plants. They play a crucial role in plant adaptation to stress and changing environmental conditions. The extremely low concentrations in which phytohormones occur present a challenge for their direct analysis and quantification, which has only been overcome thanks to recent advances in mass spectrometry. However, our knowledge of the dynamic distribution of phytohormones at the subcellular level remains incomplete. For these reasons, it is essential to develop new methodological approaches that would enable us to improve the results of subcellular fractionation and isolation.
To achieve this, we developed Fluorescence-Activated multi-Organelle Sorting (FAmOS)[1], a novel subcellular fractionation technique based on flow cytometry (FACS). FAmOS enables the simultaneous sorting of four differently labeled organelles from a whole plant, based on light scatter and fluorescence parameters while preserving phytohormone metabolic stability.
FACS was also employed to sort protoplasts obtained from GFP labeled cell populations of the Arabidopsis root tip. With the combination of LC-MS/MS, we are able to quantify phytohormones in each of isolated organelle and protoplast fractions.
Our goal is to map the intracellular distribution of phytohormones across key organelles—nucleus, chloroplasts, mitochondria, and endoplasmic reticulum—in distinct cell types of the Arabidopsis root tip. To achieve this, we employ a "double-sorting" strategy that integrates protoplast isolation with the FAmOS method. This approach enables us to uncover distinct organelle-specific phytohormone localization patterns and reveal a comprehensive view of subcellular hormone dynamics.

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Exploring the potential of microalgal extracts for mitigating salt stress in tomato plants

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Soil salinization is an escalating global issue that significantly limits crop productivity by disrupting water uptake and essential metabolic processes. Microalgal aqueous extracts (MAEs), enriched with osmoprotectants, antioxidants, and signaling molecules, have emerged as promising biostimulants to enhance plant resilience to abiotic stress. This study evaluated the potential of MAEs derived from five microalgal strains—cultivated under autotrophic or mixotrophic conditions—to mitigate salt stress in tomato (*Solanum lycopersicum* L. cv. 'Moneymaker'). FT-IR spectroscopy revealed distinct biochemical profiles of lipids, proteins, and carbohydrates depending on the growth conditions. Germination assays confirmed the absence of phytotoxic effects across all MAEs, with those from autotrophic cultures significantly enhancing germination rates ($p < 0.05$). In hydroponic trials conducted under control (1 mM NaCl) and saline (80 mM NaCl) conditions, the extract from autotrophically grown *Chlorella* spp. (#124) significantly improved plant survival (from 50% to 85%) and increased shoot biomass by 117.24% ($p < 0.01$). A five-week foliar application trial further validated these findings, demonstrating enhanced survival, chlorophyll content, photosynthetic efficiency (Fv/Fm), and biomass accumulation under salt stress. These results underscore the potential of *Chlorella*-based MAEs in mitigating salinity-induced damage and improving plant performance. Ongoing transcriptomic and phytohormonal analyses aim to elucidate the molecular pathways involved in MAE-induced salt tolerance, guiding the development of microalgae-based biostimulants for sustainable agriculture in saline environments.

Using hormone biosensors to investigate the response to osmotic stress

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Phytohormones play crucial roles in plant growth and adaptation to various stresses. In our work we are focused on the effects of drought and osmotic stress in the early stages of seedling development and their effect on physiology and phytohormone levels, mainly gibberellin (GA) and abscisic acid (ABA).
GA is an important growth hormone which promotes germination, growth of developing tissues as well as flowering[1]. In addition, GA plays an important role in the stress response, including the redistribution of growth between leaves to roots that occurs in response to drought and osmotic stress [2, 3].
We are using *Arabidopsis thaliana* to better understand the involvement of GA signaling in the response to osmotic stress and in the communication between roots and shoots. We are using polyethyleneglycol (PEG) to induce drought/osmotic stress *in vitro* with transcriptomics, hormonomics, gene reporters and GA-metabolism mutants to investigate the role of GA in response to this stress. To determine the effect on GA and ABA content and distribution with high spatial resolution we are using the Gibberellin Perception Sensor (GPS2) and the ABA sensor ABACUS2, which are based on the hormone receptors[3].
This talk will describe the use of these biosensors and present some preliminary results from *Arabidopsis* seedlings exposed to PEG.

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Sugar flow under control: A new era in agriculture

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What if we could manipulate sugar flow? Sugars play an irreplaceable role in plant metabolism - not only as an energy source, but also as signaling and building molecules essential for other biochemical processes. This makes sugar transport a fundamental process in plant physiology. Moving sugars from source tissues to sink tissues is a complicated and tightly regulated system. This research ultimately aims at the regulation and manipulation of sugar transport, which has the potential to enhance plant resilience and nutritional value.
We recently discovered CANAR, the first receptor identified that can regulate sugar transport from source to sink tissues in plants. CANAR offers a new level of control by adjusting the rate and destination of sugar transport. Additionally, the long non-coding RNA CARMA fine-tunes the expression of CANAR in the phloem, forming a regulatory module that responds to osmotic changes. At this point, the exact molecular mechanism of CANAR effect on sugat transport is still unknown. Our strategy involves generating transgenic lines with altered CANAR expression and observing their phenotypes under controlled conditions. The precise knowledge of molecular components of CANAR signalling pathway is an essential prerequisite for developing next generation tools to boost crop yield.

Development of coupled gas chromatography and photoacoustic detection methods for measuring endogenous ethylene in plants

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We created a method based on coupled gas chromatography (GC-FID) and photoacoustic detection (ETD-300) for measurement of ethylene production in plants with induced endogenous levels. Both of these types of devices have been used in the past to measure ethylene production in plants, but a detailed comparison of the results of these approaches and an attempt to merge them into a single method have not yet been published. Our aim was to develop a method, that would combine the advantages of both approaches. While the use of GC has been proven for a long time to be very effective and requiring small amounts of sample gas, photoacoustic detection provides more sensitive alternative. Our method, unlike typical methods using photoacoustic ethylene detection, involves measuring plant fresh mass and thus we determine the results per unit fresh plant weight. This allows us to recognize whether the change in ethylene production was due to an increase in plant fresh

weight induced by the test compound and/or whether the treatment actually affects ethylene production per se. Our method is also unique in that unlike typical methods where plants are grown on treated media, we grow them on untreated media and the treatment is applied at a precise time interval. This allows us to eliminate the effect of the treatment on plant germination and thus we can more accurately simulate the effect of the treatment under field conditions. Another advantage is that the subsequent use of the two methods on the same sample allows us to recognize machine errors that would otherwise be attributed to the biological variability. Because of this approach, we are also able to save the samples for further hormonal measurements by mass spectrometry. The method has been successfully tested for use with Arabidopsis plants treated with compounds known from the literature to affect ethylene production (ACC - see attached graphs, AVG, AIB, ethephon and others). Although the machines do not provide numerically identical values, the observed trends are identical and the values are in the same order.

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Transcriptomic analysis of aquaporin gene expression in response to various light conditions and abiotic stress during tomato seed germination

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In plant seeds, aquaporins control the specific transport of water and some other small molecules across membranes and are involved in various physiological processes. In tomato genome 47 aquaporin genes are present, some of them might be pseudogenes or expressed in very specific conditions. There are currently five major aquaporin gene subfamilies recognized in plants based on sequence similarities. Aquaporins have been proven to participate in responses to abiotic stresses (for example drought, salinity and temperature stress). These factors also influence aquaporin gene expression, mainly *PIPs* and *TIPs*. Interestingly, the expression of aquaporins can be also modulated by irradiance and their expression patterns strongly depends on wavelength of visible light.
Seed germination is the physiological process which is influenced by light. Although very little is known about perception of light by seeds, it was shown that in tomato blue light mostly reduces seed germination. On the contrary, this effect wasn´t observed in red light. Based on previous results, when we studied the effect of light and aquaporin-blockers ($HgCl_2$) on the tomato seeds germination, we now focused on aquaporin gene expression analysis to uncover the mechanisms of blue light-induced reduction of tomato seed germination in several tomato cultivars. The second part of our research was focused to the regulation of aquaporin transcription under treatment of $HgCl_2$ or NaCl. For analysis of gene expression in tomato we chose RNA sequencing, because it is very robust method which provides information about presence and absolute quantity of every transcript. In addition, for aquaporin genes showing significant changes during the experimental conditions, the expression was verified by qRT-PCR. Next goal of our project is to verify these finding at protein level.

The study of the effects of heavy metals on the astrocyte-like cells

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Neurodegenerative diseases, such as Alzheimer's and Parkinson's, remain among the most serious medical challenges of our time. Despite extensive research, we still lack effective treatments and a clear understanding of their underlying causes. While genetic predispositions play a role, many cases are idiopathic and believed to result from multifactorial influences, including environmental factors, exposure to toxins, genetic variability, and lifestyle[1].

In recent years, neuroinflammation has gained increasing attention. This complex biological response, originally intended to protect the CNS, can itself become a contributor to disease pathology[1]. Heavy metals, such as copper and manganese, are known to induce various pathological effects, including triggering neuroinflammatory processes[2,3]. Their impact on the CNS has been intensively studied and forms the core focus of this work.The primary aim of this work was to establish an *in vitro* cellular model of heavy metal-induced neuroinflammation. The human glioblastoma cell line U-87MG was used, as it can be differentiated into an astrocyte-like phenotype, representative of one of the brain's key immune cell types, via treatment with all-*trans* retinoic acid. Cells were exposed to a range of concentrations of copper and manganese, and multiple parameters were assessed, including reactive oxygen species (ROS) production, nitric oxide (NO) release, cell viability, mitochondrial function, and morphological alterations. Based on the results, a working hypothesis regarding the differential effects of the two metals was proposed. Subsequently, a suitable copper concentration was selected for testing of selected compounds for their potential protective effects. Of the three candidate molecules, BS224 showed promise as an astrocytoprotective agent.

This research also envisages the development of further experiments that will provide a deeper understanding of the processes involved and aims to create a model for the study of neuroinflammation which will serve as a tool for testing new, potentially protective agents.

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Novel selenium-based compounds and their role in cytoprotection against ferroptosis

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Ferroptosis, a recently characterized form of regulated cell death, is driven by iron-dependent lipid peroxidation, glutathione depletion, and redox imbalance. Unlike apoptosis, it engages distinct metabolic pathways, making it a promising target in inflammatory diseases, cancer, aging, and wound healing.
Given the high susceptibility of skin and retinal epithelial cells to oxidative and environmental stress—owing to their intrinsic ROS production and lipid-rich membranes—modulating ferroptosis in these models could offer novel strategies to maintain cell health.
Selenium-containing compounds, particularly those mimicking glutathione peroxidase (GPx) activity like ebselen, present an attractive therapeutic approach. This project investigates the cytoprotective effects of novel organoselenium compounds, expected to function as GPx mimetics, against ferroptosis-induced cytotoxicity.
We employed human dermal fibroblasts (BJ) and retinal pigment epithelial cells (ARPE-19) as models of oxidative stress and age-related degeneration. Ferroptosis was induced using RSL3, erastin, and ML-210—agents that inhibit GPx4 or alter cystine uptake. Cytoprotection was assessed via resazurin-based viability assays alongside complementary biochemical evaluations such as the GPx activity assay.Preliminary results indicate promising protective effects of the novel compounds. Future research will focus on their impact on mitochondrial supercomplex stability under ferroptotic stress and employ lipid peroxidation probes to directly visualize and quantify lipid peroxidation and its suppression.

The endoplasmic reticulum: master regulator of auxin metabolism and transport

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Auxin homeostasis is crucial for proper plant growth and adaptation to environmental cues. While the role of plasma membrane-localized transporters in polar auxin transport is well established, increasing evidence highlights the endoplasmic reticulum (ER) as a central intracellular hub for auxin regulation. ER-localized transporters, including PIN5, PIN6, PIN8, and members of the PIN-LIKES (PILS) family, contribute to the subcellular distribution of indole-3-acetic acid (IAA), the principal active auxin in plants. These proteins facilitate the sequestration or release of auxin into or from the ER lumen, thereby modulating cytosolic auxin levels and downstream signaling pathways. Additionally, the ER has emerged as a site of auxin biosynthesis and metabolism, further underscoring its integrative role in maintaining auxin equilibrium. Despite these advances, the molecular mechanisms underlying ER-mediated auxin regulation remain largely unexplored.

To advance the functional analysis of ER-localized PIN proteins in auxin homeostasis, we developed novel inducible transgenic lines of *Arabidopsis thaliana*. These lines enable controlled, temporal expression of selected

ER-resident PINs, providing a robust system for dissecting their specific contributions to intracellular auxin distribution and dynamics. This approach offers a powerful tool for investigating the influence of ER-based auxin transport on hormone gradients, signaling networks, and developmental outcomes under both normal and stress conditions. Ongoing studies using these lines aim to elucidate the mechanistic basis of ER-mediated auxin regulation and its broader physiological significance.

Different expression of CDK4 and CDK6 among b-cell non-hodgkin lymphomas as a marker of sensitivity to CDK4/6 inhibitors

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Targeting the cell cycle machinery, whose dysfunction is a major driver of tumorigenesis, represents a rational approach in cancer therapy. Inhibition of CDK4 and CDK6 kinases has radically changed the clinical perception of the treatment of advanced hormone receptor-positive breast cancer with four FDA-approved CDK4/6 inhibitors (CDK4/6i), palbociclib, ribociclib and abemaciclib. However, several tumour types possess only modest or no therapeutic benefit and biomarkers that could predict their sensitivity remain uncertain. Herein, we investigated the antiproliferative effects of palbociclib, ribociclib and abemaciclib on a panel of 50 different lymphoma cell lines representing different subtypes with the aim of expanding tumours that may benefit from CDK4/6i therapy. To our surprise, the protein expression levels of CDK4 or CDK6 differed among each disease category. Our data showed that CDK4 is predominantly expressed only in mantle cell lymphomas contrary to the expression of CDK6. The distribution of CDK4/6 expression varied in DLBCL cell lines. While most of ABC subtypes expressed the same protein levels of both proteins, GCB subtypes showed higher expression of CDK6. Members of the Burkitt lymphomas also showed predominantly higher protein levels of CDK6. Newly, we discovered that the observed differences in cell line sensitivity to ribociclib correlate with the intrinsic expression levels of CDK4 and CDK6. Cells expressing low levels of CDK6 exhibited exquisitely high sensitivity, whereas those expressing both CDK4 and CDK6 showed little or no response to ribociclib.
To investigate, whether the overexpression of CDK4 or CDK6 would alter the sensitivity of lymphoma cell lines, we established doxycycline-inducible CDK4/CDK6 overexpressing cell lines using the Sleeping beauty transposable system. We showed that doxycyclin-inducible CDK4 overexpression significantly increased the sensitivity of lymphoma cells to ribociclib, whereas CDK6 overexpression did not. The obtained results demonstrated that low CDK6 expression may predict sensitivity to ribociclib and thus may serve as a useful biomarker to stratify patient response to CDK4/6i therapy. Additionally, retinoblastoma protein deficiency was confirmed as the next determinant of intrinsic resistance to CDK4/6 inhibition. In addition, for those types of lymphomas with poor outcomes with CDK4/6i treatment, we proposed novel treatment settings represented by combined therapy with PI3K inhibitors such as idelalisib and alpelisib, or with ERβ modulators, tamoxifen and acolbifene, providing promising results for the future improvement in lymphoma therapy.

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Analysis of the barley and its mutant *chlorina f2*^{f2} chloroplast proteome

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Barley (*Hordeum vulgare*) is not only one of the most important cereal crops, but also an important model organism for studying chloroplasts and their substructures. Its suitability for this research is further enhanced by the availability of numerous mutant lines. One of the most widely used mutants is *chlorina f2*^{f2}, which is characterized by a strongly reduced content, or complete absence, of chlorophyll b. *chlorina f2*^{f2} has played a crucial role in numerous studies investigating the importance of chlorophyll and the effects of its deficiency on the function of photosynthetic complexes, thylakoid organization, chloroplast structure, and overall plant physiology. Interestingly, no complex proteomic study on *chlorina f2*^{f2} has yet been published despite this fact. In this study, we isolated chloroplasts from seven-day-old wild-type barley cultivar Morex leaves and the *chlorina f2*^{f2} mutant. Thylakoid membranes were subsequently extracted from these chloroplasts, and comprehensive proteomic analyses of both compartments were performed. Our results revealed a significant downregulation of proteins involved not only in photosynthesis, chlorophyll biosynthesis, and thylakoid organization, findings that are consistent with previously reported physiological and biochemical characteristics of the *chlorina f2*^{f2}, but also in several hormone biosynthetic pathways. Follow-up analyses of phytohormone levels and key metabolites further supported these findings. Taken together, our data significantly expand the current understanding of *chlorina f2*^{f2} physiology. Rather than merely a model for chlorophyll b deficiency, *chlorina f2*^{f2} should be considered a more complex system that also reflects alterations in plant hormonal homeostasis and stress-related signalling pathways. From this point of view, it represents a valuable resource for future studies focused on the interplay between pigment biosynthesis, photosynthetic efficiency, and hormonal regulation in plants.