CHEMISTRY AND BIOLOGY OF PHYTOHORMONES AND RELATED SUBSTANCES

2026

May 18-20, 2026 – Hotel Jelenovská, Valašské Klobouky, Czech Republic

Abstract Book

Laboratoř růstových regulátorů
Laboratory of Growth Regulators
www.rustreg.upol.cz

Faculty of Science

Institute of Experimental Botany of the AS CR, v. v. i.

CZECH SOCIETY OF EXPERIMENTAL PLANT BIOLOGY

Sponsors


BIO-RAD


bioTech
innovative


Elisabeth®
Pharmacon


CHROMSERVIS


LABO
SERV


labmark


Schoeller
INSTRUMENTS


GeneProof
part of NuvinkaDx


vwr
part of avantor


Waters


KRD
solutions for life science


IChemIm Ltd

SMART
Plant Biotechnology
for Sustainable Agriculture

The conference is supported from European Regional Development Fund-Project „SMART Plant Biotechnology for Sustainable Agriculture“ (No. CZ.02.01.01/00/23_020/0008497).
https://lgr.upol.cz/smart-projekt/

CHEMISTRY AND BIOLOGY OF PHYTOHORMONES AND RELATED SUBSTANCES 2026

https://lgr.upol.cz/cbprs2026

Co-funded by
the European Union

MINISTRY OF EDUCATION,
YOUTH AND SPORTS

--- ABSTRACT BOOK ---

Development of a new method for the detection of enzymatic oxidation of auxins and related compounds

Benda M[1], Bělíček J[2], Pěnčík A[3], Žukauskaitė A[1], Brunoni F[4]

[1]Department of Chemical Biology, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

[2]Department of Experimental Biology, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

[3]Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences & Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

[4]Department of Biotechnology, University of Verona, Strada le Grazie 15, 37134, Verona, Italian Republic.

E-mail: marek.benda01@upol.cz

Auxins, primarily indole-3-acetic acid (IAA), are essential phytohormones that regulate plant growth and development. In *Arabidopsis thaliana* (*At*), IAA homeostasis is largely maintained through reversible amino acid conjugation and irreversible oxidation, the latter mediated by dioxygenase for auxin oxidation 1 (DAO1). As DAO1 preferentially acts on IAA-amino acid conjugates rather than free IAA, defining its substrate specificity can prove useful for understanding auxin turnover[1,2]. While mass spectrometry provides high sensitivity and selectivity, spectrophotometry offers a well-established and cost-effective alternative for enzyme activity assays.

Using recombinant *At*DAO1, we developed a spectrophotometric assay based on the consumption of its co-substrate 2-oxoglutarate. Following derivatization with 2,4-dinitrophenylhydrazine, the resulting hydrazone was quantified at a 425-nm wavelength[3]. The reaction conditions were optimized for buffer composition (sodium phosphate), pH (optimum at 7.4), and reducing agent (L-ascorbic acid).

Application of this assay to a library of synthesized IAA-amino acid conjugates confirmed previously reported oxidation of IAA-Ala, IAA-Asp, IAA-Glu, and IAA-Leu by *At*DAO1[1,2]. In addition, oxidation of IAA-Ile, IAA-Phe, IAA-Trp, and IAA-Val was observed. Validation by HPLC–MS/MS demonstrated good precision (mean CV = 9%) and acceptable trueness (mean RE = 21%) for IAA conjugates. These findings indicate a broader substrate specificity for *At*DAO1 than previously recognized and establish a rapid, high-throughput approach for screening novel substrates of auxin-oxidizing enzymes.

The authors acknowledge financial support from the project TowArds Next GENeration Crops (TANGENC) (reg. no. CZ.02.01.01/00/22_008/0004581), funded by the ERDF Programme Johannes Amos Comenius, and from the Internal Grant Agency of Palacký University Olomouc (IGA_PrF_2025_013, IGA_PrF_2025_019, and IGA_PrF_2026_013).

References

[1] **Hayashi K. et al. 2021.** Nature Communications 12: 6752.
[2] **Müller K. et al. 2021.** Plant Physiology 187(1): 103–115.
[3] **Wong S. et al. 2021.** Journal of Biological Chemistry 296: 100397.

Study of salicylic acid metabolism in plants using mass spectrometry

Budíková B[1], Široká J[1], Žukauskaitė A[2], Novák O[1]

[1]Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences & Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

[2]Department of Chemical Biology, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

E-mail: beata.budikova@upol.cz

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 signaling pathways leading to processes that help plants to defend themselves. The defense signaling 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 defense mechanisms in plants. Defense 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 defense 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.

The authors would like to acknowledge the support of the grant IGA_PrF_2026_014.

A Novel LC-MS/MS Approach for Investigating *S*-adenosylmethionine Metabolism in Plants

Cermanová K[1,2], Bublavá P[1,2], Darbandsari M[1,2], Novák O[1,2], Karady M[1,2]

[1]*Laboratory of Growth Regulators, Faculty of Science, Palacký University, Šlechtitelů 27, 779 00, Olomouc, Czechia*

[2]*Laboratory of Growth Regulators, Institute of Experimental Botany, The Czech Academy of Sciences, Šlechtitelů 27, Olomouc, Czechia*

E-mail: katerina.cermanova01@upol.cz

The Yang cycle, also known as the methionine cycle or methionine salvage pathway, is a fundamental metabolic pathway present in plants as well as in many eukaryotes and prokaryotes. Its central molecule, *S*-adenosyl-L-methionine, functions as a universal methyl group donor in numerous enzymatic reactions. More importantly, it is also a precursor for polyamine and ethylene biosynthesis in plants. While the metabolic pathways of these molecules are well elucidated, comprehensive and validated profiling methods for their detection in plants remain limited.

Therefore, our research focused on the development of novel UHPLC–MS/MS methods for the quantification of these molecules. First, a method enabling simultaneous quantification of ethylene precursor 1-aminocyclopropane-1-carboxylic acid together with phytohormones and related compounds from a single plant extract was established. Subsequently, this platform was extended to incorporate polyamines and related compounds, including amino acid intermediates.

The method is optimized for 10 mg of fresh weight plant material, incorporating a minimal extraction protocol. Application of this approach to *Arabidopsis thaliana* and *Solanum lycopersicum* seedlings revealed species-specific metabolic responses to abiotic stresses, with drought and salinity triggering distinct adjustments in polyamine and ethylene precursor levels and spermine exhibiting stress-specific fluctuations.

The authors would like to acknowledge the support provided by The Czech Science Foundation (GAČR) via 20-25948Y junior grant, by Internal Grant Agency of Palacký University (IGA_PrF_2025_019), Jaroslav Tupý Endowment Fund, and by the "Biorefining and circular economy for sustainability" (TN02000044) grant.

Effect of stress on ethylene and other phytohormone levels in Arabidopsis

Darbandsari M[1], Karady M[1], Strnad M[1]

[1]Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences & Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

E-mail: mersa.darbandsari01@upol.cz

Ethylene, a vital gaseous phytohormone, orchestrates a myriad of plant developmental processes, prominently including responses to environmental stresses. This phytohormone not only regulates growth and senescence but also plays a crucial role in mediating plant responses to abiotic stresses such as drought, salinity, and temperature extremes.

By modulating gene expression and signaling pathways, ethylene enhances plant resilience and adaptability, thereby influencing overall crop productivity. 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, which is essential for ensuring food security in an increasingly volatile environment[1,2].

We aim to how altered ethylene production in Arabidopsis mutants affects the profiles of various phytohormones, especially under abiotic stress conditions. our research employs a validated derivatization method to precisely measure levels of ethylene's precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), alongside other phytohormones. Our findings reveal significant interactions between ethylene, ACC, and other phytohormones, highlighting how changes in ethylene production can influence overall hormonal balance and stress response mechanisms in plants. This research underscores the potential of advanced analytical methods for comprehensive phytohormone profiling, providing insights that could lead to the development of crops with enhanced stress tolerance and optimized growth.

References

[1] **Lin, Zhefeng, et al. 2009.** Journal of experimental botany 60.12: 3311-3336.
[2] **Zhang, Huiming, et al. 2022**. Nature Reviews Genetics 23.2: 104-119.

Seaweed extract alleviates nutrient deficiency in cucumber seedlings under hydroponic conditions

Filipský I[1,3,4], Kulkarni MG[1,2], Gupta S[2,3,4], Doležalová I[3,5], Van Staden J[2], Novák O[3,4] and Doležal K[1,4]

[1]*Department of Chemical Biology, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.*

[2]*Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa*

[3]*Laboratory of Growth Regulators, Faculty of Science, Palacký University, Šlechtitelů 27, 78371 Olomouc, Czech Republic*

[4]*Laboratory of Growth Regulators, Institute of Experimental Botany AS CR, v.v.i, Šlechtitelů 27, 78371 Olomouc, Czech Republic*

[5]*Czech Agrifood Research Center, Šlechtitelů 892/29, 779 00 Czech Republic*

E-mail: ivan.filipsky@upol.cz

Nutrient deficiencies in hydroponic systems limit crop performance and increase dependence on chemical fertilizers. Seaweed-based biostimulants represent a promising strategy for improving plant resilience under suboptimal nutrient conditions. This study evaluated the potential of the seaweed extract Kelpak®, derived from *Ecklonia maxima*, to alleviate nutrient stress in hydroponically grown cucumber (*Cucumis sativus* L.) seedlings. Four Kelpak concentrations (0, 0.5, 1.0, and 1.5%) were tested under two nutrient-stress models: reduced-strength Hoagland's solution (0–15%) and macronutrient-specific deficiencies (50% Hoagland's solution lacking N, P, or K).

Kelpak application significantly enhanced shoot and root growth, leaf number, and biomass accumulation under low-nutrient and potassium-deficient conditions, with the most consistent responses observed at 0.5% and 1.0%. Moderate improvements in photosynthetic pigment content and stress-related proline accumulation indicated enhanced physiological adaptation to nutrient limitation. In contrast, responses were limited under severe nitrogen deficiency. Carbohydrate and protein contents showed no consistent trends in response to Kelpak treatment.

Nutrient analyses revealed treatment-dependent effects on mineral accumulation, including positive associations between Kelpak concentration and potassium content under potassium deficiency, as well as variable effects on calcium, magnesium, and micronutrients. Overall, Kelpak improved seedling performance primarily under moderate nutrient stress.

These findings demonstrate that Kelpak can partially compensate for reduced nutrient availability and support sustainable hydroponic production by reducing reliance on high fertilizer inputs. Further research is warranted to clarify long-term effects and optimize application strategies.

The authors would like to acknowledge the support of the University of KwaZulu-Natal and the National Research Foundation, South Africa as well as the project TowArds Next GENeration Crops, reg. no. CZ.02.01.01/00/22_008/0004581 of the ERDF Programme Johannes Amos Comenius, Palacký University (IGA_PrF_2026_014) and by the Ministry of Agriculture of the Czech 438 Republic, institutional support MZE-RO0425.

Exploring tetrahydro-3*H*-pyrazolo[4,3-*f*]quinolines for tubulin-targeted anticancer therapy

Kollárová V[1], Dayal N[2] Yadav P[3], Peřina M[1], Bělíček J[1], Vojáčková V[1], Krňávková P[1], Jorda R[1], Sintim HO[3]

[1]*Department of Experimental Biology, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.*

[2]*Department of Chemistry, Purdue University, West Lafayette, IN, 47907, USA.*

[3]*Department of Chemistry and Biochemistry, University of Notre Dame, IN, 46556, USA*

E-mail: veronika.kollarova01@upol.cz

Microtubules represent a crucial therapeutic target in anticancer therapy, however, the clinical use of many tubulin modulators is limited by toxicity and the development [1]. In this study, a series of tetrahydro-3*H*-pyrazolo[4,3-*f*]quinoline analogues, derived from the lead compound HSD1787, were synthesized via a single-flask Povarov multicomponent reaction and evaluated as a promising class of small molecules that potently inhibited the NCI-60 cancer cell panel at submicromolar concentrations by targeting tubulin polymerization and disrupting mitotic progression. Initial NCI-60 COMPARE analysis indicated that the growth inhibition profiles of HSD1787 closely resembled those of known tubulin polymerization inhibitors, prompting further experimental validation[2, 3]. Functional assays subsequently demonstrated that HSD1787 interferes with tubulin polymerization in a manner similar to colchicine. Building on this result, the present study focused on the design and evaluation of its analogues. Among the tested tubulin modulators, several compounds exhibited high potency and induced a marked G2/M cell-cycle arrest, warranting further investigation of their potential application in cancer therapy. These findings were further supported by immunofluorescence data showing tubulin reorganization in HeLa cells, as well as by Western blot analysis of key mitotic and apoptosis markers.

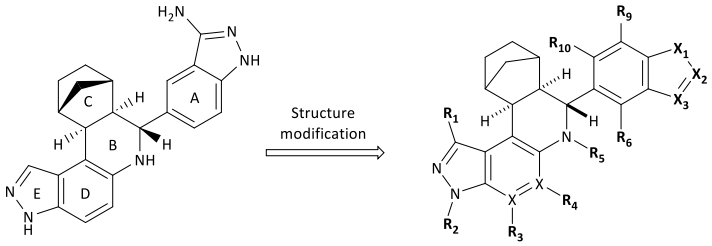

Figure 1. Design strategy based on structural modification of the lead compound to obtain tubulin-modulating analogues.

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.

References

[1] **Peřina M et al. 2025.** Arch. Pharm (Weinheim). 358(7):e70031 .
[2] **Dayal N. et al. 2020.** ACS Omega 5, 23799–23807.
[3] **Sintim H. O. et Dayal N. 2023.** U.S. Patent Application US20230312562A1.

Towards improved *in vitro* models for neurodegenerative disease drug discovery

Kordinová H[1], Gonzalez G[2], Kvasnica M[1]

[1]Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences & Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

[2]Department of Neurology, University Hospital Olomouc and Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic.

E-mail: helena.kordinova@upol.cz

Neurodegenerative diseases are a heterogeneous group of disorders characterized by progressive neuronal loss, leading to cognitive and motor dysfunction[1]. The most common forms, including Parkinson's and Alzheimer's diseases, are becoming increasingly prevalent with population aging, representing a growing global health burden. Despite extensive research, current therapeutic strategies remain largely symptomatic, underscoring the urgent need for disease-modifying approaches. The multifactorial nature of NDs, involving genetic, environmental, and cellular factors, further complicates their study and treatment.

Our work is aimed at the discovery and characterization of novel compounds with potential neuroprotective properties. To assess their biological activity, we employed the SH-SY5Y human neuroblastoma cell line, differentiated using 10 µM all-trans retinoic acid to promote neuron-like features[2]. While this model is advantageous due to its robustness and reproducibility, its tumor-derived nature restricts its ability to fully replicate physiological neuronal conditions. To address this limitation, we are in the process of establishing a more physiologically relevant *in vitro* system based on neuronal stem cells[3]. This advanced model is expected to better reflect human neuronal biology and disease mechanisms, particularly when combined with targeted stressors and neurotoxic agents, thereby enabling a more accurate evaluation of neuroprotective effects.

Overall, this study contributes to the development of improved experimental models for neurodegeneration research and supports the ongoing search for effective neuroprotective agents.

The authors would like to acknowledge the support of the grant IGA_PrF_2026_014.

References

[1] **Gadhave D. G. et al. 2024**. Ageing Research Reviews 99(102357).
[2] **Kordinová H. et al. 2026**. European Journal of Medicinal Chemistry 301(118217).
[3] **Ottoboni L. et al. 2020**. Frontiers in Neurology 11(148).

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

Kubaláková B[1], Bryksová M[1], Plíhalová L[1]

[1]Department of Chemical Biology, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

E-mail: beata.kubalakova01@upol.cz

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 current results of the created BEK series. In the experimental part biological and chemical methods are listed and specific methods of chemical synthesis for the preparation N6-substitued kinetin derivatives and analogues of 6-substituted purine-9-D-mannopyranosides and purine-9-L-rhamnopyranosides are approximated and characterized.

*The authors would like to acknowledge the support of the grants [*CZ.02.01.01/00/23_021/0008909*] and [OP JAK] from [MŠMT].*

CRISPR induced mutation of phytohormone amido-synthetase GH3B in *Marchatia polymorpha*

Kuštović I[1], Vladejić J[2], Brunoni F[3], Novák O[1,2]

[1]*Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences & Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.*

[2]*Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences & Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic*

[3]*Department of Biotechnology, Faculty of Science, University of Verona, Str. le Grazie, 15, 37134 Verona, Italy.*

E-mail: kustiv00@upol.cz

Plant hormones are signaling molecules that regulate nearly all aspects of plant growth and development. Although significant progress has been made in understanding hormone perception, signaling and synthesis, the mechanisms that maintain hormone homeostasis remain less clearly understood[1]. An important mechanism by which plants control the hormone abundance and activity is conjugation to amino acids, a reaction catalyzed by the members of the GRETCHEN HAGEN 3 (GH3) protein family[2]. However, the role hormone conjugation has so far been studied mainly in angiosperms. Our project aims to investigate the function and importance of GH3-mediated hormone conjugation in liverwort *Marchantia polymorpha,* an early diverging land plants. *M. polymorpha* is a valuable model for studying of land plants evolution due to its basal phylogenetic position, low genetic redundancy and relative simplicity of genetic manipulation[3]. Two *GH3* gene variants, *GH3a* and *GH3b*, were identified in *M. polymorpha*. Previous research[4] showed that the basal activity of the GH3A enzyme is sufficient for most conjugation of dn-OPDA (dinor-12-oxo-phytodienoic acid), a molecule involved in the jasmonate signaling pathway and plant responses to stress. Nevertheless, the detection of low levels of conjugates in mutants without functional *GH3a* points to the existence of an additional enzyme involved in this process, which opens the possibility that the GH3b variant has a complementary role. To clarify the role of the GH3b we are generating mutants using the CRISPR/Cas9 genome editing method, in order to create mutants lacking *GH3b* and both *GH3a* and *GH3b*[5]. Following the mutant establishment, hormone profiling will be performed to check *in vivo* contribution of GH3b to hormone conjugation in *M. polymorpha*.

References

[1] **Porco S. et al. 2016.** Proceedings of the National Academy of Sciences 113(39): 11016-11021.
[2] **Müller K. et al. 2021.** Plant Physiology 187: 103–115.
[3] **Ishizaki K. et al. 2016.** Plant and Cell Physiology 57(2): 262-270.
[4] **Liang W. et al. 2024.** preprint bioRxiv.
[5] **Sugano SS. and Nishihama R. 2018**. Plant Transcription Factors: Methods and Protocols: 109-126.

Flowing with plants: Unlocking secrets with Cytometry

Lemberk J[1], Hladík P[1], Skalický V[2], Pěnčík A[1], Lenobel R[1], Novák O[1]

[1]Laboratory of Growth Regulators, Institute of Experimental Botany, Czech Academy of Sciences & Palacký University Olomouc.

[2]Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences

Email: jakub.lemberk@upol.cz

Plant hormones, or phytohormones, are naturally occurring compounds that act as bioactive signaling molecules, influencing plant growth, development, and stress responses. Their very low concentrations make direct analysis challenging, despite recent advances in mass spectrometry and purification techniques[1], and their subcellular distribution remains poorly understood. Here, fluorescence-activated cell sorting (FACS) was first used to isolate protoplasts from fluorescently labeled root tip cells of Arabidopsis thaliana[2]. In a separate experiment, organelles were isolated from whole plants using FACS with specific fluorochromes[3].

We are now working to combine these approaches into a "double sorting" workflow, linking cell-type-specific protoplast isolation with organelle-level separation to extract and quantify phytohormone profiles and map their distribution at the organelle level in root tips. This strategy will help elucidate phytohormone transport and metabolism and deepen understanding of their roles in cellular processes.

This work was supported by the European Research Council (ERC) under the Horizon Europe programme (reg. no. 101168880, STARMORPH), the ERDF programme Johannes Amos Comenius: TowArds Next GENeration Crops (reg. no. CZ.02.01.01/00/22_008/0004581), and the Internal Grant Agency of Palacký University (project IGA_PrF_2026_014).

References

[1] **Novák O., et al. (2017):** Annu Rev Plant Biol. 68: 323–48.
[2] **Petersson S.V., et al. (2009):** Plant Cell 21: 1659–1668.
[3] **Skalický V., et al. (2023):** The Plant Journal, 116: 1825-1841.

Synthesis and biological activity of C2-substituted purine derivatives

Mastná J[1,2], Mik V[3], Zatloukal M[1], Vylíčilová H[1], Humplík J[1], Voller J[3,4], Strnad M[2]

[1]Department of Chemical Biology, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

[2]Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences & Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

[3]Department of Experimental Biology, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

[4]Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine and Dentistry, Palacký University Olomouc, Hněvotínská 5, 779 00, Olomouc, Czech Republic.

E-mail: jana.mastna01@upol.cz

Cytokinins, a major class of plant hormones primarily recognized for their role in promoting cytokinesis and regulating numerous aspects of plant growth and development, have been extensively studied since their discovery in the 1950s[1]. Our research introduces a novel group of cytokinin analogues: C2-substituted purines (so called *iso*-cytokinins), which have remained largely unexplored in both synthetic chemistry and biological evaluation.

A series of 28 isoprenoid and aromatic *iso*-cytokinins were synthesized and subsequently evaluated using standard cytokinin bioassays (tobacco callus growth, wheat leaf senescence, and root inhibition assays), as well as receptor-binding assays (AHK3 and AHK4 receptor assays, along with *ARR5::GUS* assay). Several *iso*-cytokinins displayed moderate cytokinin-like activity without the pronounced inhibitory effects typically observed in natural cytokinins. Notably, these compounds did not activate cytokinin receptors, indicating that their biological effects are likely mediated through alternative biochemical pathways. In parallel, their potential of having antioxidant properties was assessed using ABTS (TEAC) and FRAP assays, with compounds 11 and 25 demonstrating antioxidant capacities comparable to that of the reference compound Trolox in the ABTS assay. The combination of low cytotoxicity, moderate biological activity, and measurable antioxidant potential suggests possible applications in fields such as the cosmetic industry, where higher local concentrations may be utilized. Overall, this work establishes a basis for further investigation of C2-substituted *iso*-cytokinins, particularly regarding their bioactivity, receptor-independent mechanisms, and potential applications.

The authors would like to acknowledge the support of the grant No. CZ.02.01.01/00/23_020/0008497 and "SMART Plant Biotechnology for Sustainable Agriculture" from European Regional Development Fund-Project co-funded by the European Union and Ministry of Education, youth and sports (Czech Republic).

References

[1] **Miller, C. O. et al. 1955**. 77(5), 1392–1392.

Biosensors in determining the role of gibberellins in the response to osmotic stress in wheat and *Arabidopsis*

Miksteinová T[1], Griffith J[3], Jones A[3], Pearce S[2], Hedden P[1,2]

[1]*Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences & Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.*

[2]*Rothamsted Research, Harpenden AL5 2JQ, UK*

[3]*Sainsbury Laboratory, University of Cambridge, Cambridge CB2 1LR, UK*

E-mail: miksteinova@ueb.cas.cz

Phytohormones are key regulators of plant growth, development, and stress adaptation. In this study, we investigate the impact of osmotic stress on early seedling development, with a particular focus on gibberellin (GA) dynamics in wheat, complemented by analyses in *Arabidopsis thaliana*. Osmotic stress is induced *in vitro* using polyethylene glycol to simulate water-deficit conditions.

GAs are known to promote germination and tissue expansion and contribute to stress adaptation by reallocating resources from shoots to roots[1,2]. To dissect GA-mediated responses, we combine physiological, molecular approaches and work with mutant and reporter lines. At the molecular level, spatial dynamics of GA metabolism are resolved using the Gibberellin Perception Sensor 2 (GPS2)[3], enabling high-resolution visualization of GA distribution during stress adaptation. This approach provides insights into the regulation of GA signaling under osmotic stress conditions.

The authors would like to acknowledge the support of the IGA_PrF_2026_014; European Regional Developmental Fund to project "Towards Next Generation Crops" No. CZ.02.01.01/00/22_008/0004581(TANGENC)

References

[1] **Hedden P. et al. 2020.** PCP 61(11): 1832–1849.
[2] **Colebrook E.H. et al. 2014.** J. Exp. Biol. 217(1): 67–75.
[3] G**riffiths J. et al. 2024.** The Plant Cell 36(10): 4426–4441.

Phytohormones in biostatistics

Pavlíčková T[1], Petřík I[1], Chamrád I[1], Lenobel R[1], Novák O[1]

[1]Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences & Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic

E-mail: tereza.pavlickova02@upol.cz

Design of Experiments (DoE) represents a systematic and statistically driven alternative to conventional comparative experimentation, enabling efficient optimization of analytical methods while accounting for interactions between experimental parameters. Despite the widespread use of Python in analytical chemistry and data science, reliable and comprehensive tools for Design of Experiments (DoE) are still limited.

To address this limitation, a Python package is being developed to provide an integrated pipeline for experimental design, data processing, statistical evaluation and visualization. The aim is to create a simple framework that covers the whole experimental workflow, from experiment design to data analysis, in one environment.

The developed workflow is being applied to the development of an LC–MS method for the analysis of plant peptide hormones. Current work is focused on the peptide hormone PSK-α, where a reference standard was used to optimize the MS response. Different experimental conditions were tested to improve signal intensity and overall method performance. This application serves as a practical example of how Design of Experiments and automated data analysis can support analytical method development. The combination of statistical experimental design and computational tools can help make LC–MS optimization faster, more systematic, and easier to reproduce.

The authors would like to acknowledge the support provided by The Czech Science Foundation "Role of Indole Metabolism in Enhancing Abiotic Stress Tolerance in Brassicaceae" (No. 26-22427K), by Internal Grant Agency of Palacký University (IGA_PrF_2026_014), and European Regional Development Fund-Project "Interdisciplinary Approaches for the Development and Application of New Materials in Medical Practice - New Omic Technologies" (No. CZ.02.01.01/00/23_021/0009224).

Matrix effects: An Achilles heel of quantitative LC-MS/MS analysis of plant hormones

Petřík I[1], Vencálek O[2], Pavlíčková T[1], Široká J[1], Kaleta M[1]

[1]Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences & Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

[2]Palacky University Olomouc, Department of Mathematical Analysis and Applications of Mathematics, Olomouc, Czech Republic

E-mail: michal.kaleta@upol.cz; ivan.petrik@upol.cz

Matrix effects are a persistent source of bias in quantitative LC–MS workflows based on linear calibration and remain a central challenge in method validation and optimization. Although they are routinely evaluated using empirical descriptors such as the matrix-matched response ratio and the calibration-slope ratio, the theoretical relationship between these metrics has not been clearly defined. As a result, their interpretation may remain ambiguous, particularly when matrix effects involve both background interferences and changes in calibration slope. This work is primarily devoted to a theoretical clarification of matrix-effect assessment in linear LC–MS calibration. We derive a unified mathematical framework that links conventional matrix-effect descriptors to the parameters of the calibration model. Within this framework, matrix effects are decomposed into two independent components associated with the intercept and the slope of the calibration function. This decomposition separates concentration-independent baseline shifts from concentration-dependent proportional changes in signal response and provides a formal basis for interpreting how different matrix-effect metrics behave across the calibration range. By formally connecting commonly used empirical descriptors with regression-model parameters, the framework clarifies their meaning, limitations, and complementarity. The approach may support more rigorous interpretation of matrix effects in LC–MS-based omics applications, including metabolomics, proteomics, and lipidomics, where reliable quantification in complex biological matrices remains essential.

The authors would like to acknowledge the support of the grant TANGENC: Towards next generation crops (CZ.02.01.01/00/22_008/0004581) from Johannes Amos Comenius programme funding by Ministry of Education, Youth and Sports.

Sugar Flow Engineering: CARMA–ARR7 Tunes Sugar Responsiveness of CANAR

Slovjaková B[1], Trávníčková T[1], Sacharowski S[2], Kovačík M[3], Špundová M[4], Pečinka A[3], Swiezewski S[2], Hajný J[1]

[1]Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences & Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

[2]Laboratory of Seeds Molecular Biology, Institute of Biochemistry and Biophysics Polish Academy of Sciences, Pawinskiego 5a, 02-106 Warsaw, Poland.

[3]Center of Plant Structural and Functional Genomics, Institute of Experimental Botany, Czech Academy of Sciences, Slechtitelu 31, 77900 Olomouc, Czech Republic.

[4]Department of Biophysics, Faculty of Science, Palacky University, Slechtitelu 27, 77900 Olomouc, Czech Republic.

E-mail: barbora.slovjakova@upol.cz

Sugars are central to plant metabolism, yet the regulatory mechanisms controlling their transport remain only partially understood. In *Arabidopsis thaliana*, the receptor-like kinase CANAR coordinates auxin canalization with shoot-to-root sugar transport, influencing vascular patterning, cell turgor and size. Its sucrose-inducible expression is modulated by the lncRNA CARMA, which forms a feedback loop to adjust CANAR output[1,2].

We uncover a regulatory link between CARMA and the cytokinin response regulator ARR7. CARMA acts as a chromatin-associated lncRNA, modulates ARR7 transcription. This module limits early sucrose-driven accumulation of CANAR mRNA and, through ARR7, shapes longer-term CANAR protein levels. Together, these findings establish the CARMA–ARR7 module as a key regulator fine-tuning CANAR sugar responsiveness across temporal scales.

Understanding how sugars are allocated from source to sink tissues provides a foundation for manipulating sugar transport in an organ-specific manner, with potential to improve crop yield, stress resilience, and nutrient allocation in targeted tissues.

The authors would like to acknowledge the support of the grants JG_2024_003 implemented within the Palacký University Young Researcher Grant and by JUNIOR STAR GAČR (26-20529M). Research was partly supported by the project TowArds Next GENeration Crops (reg. no. CZ.02.01.01/00/22_008/0004581) of the European Regional Development Fund programme Johannes Amos Comenius.

References

[1] **Hajný et al. 2024**. Molecular Plant 17(11): 1719-1732.
[2] **Hajný et al. 2020**. Science 370 (6516): 550-557.

Comparative study of gas chromatography and photoacoustic detection for measuring endogenous ethylene in plants

Strnad J[1], Karady M[1], Cermanová K[1], Strnad M[1]

[1]Laboratory of Growth Regulators, Palacký University & Institute of Experimental Botany of the Czech Academy of Sciences, Šlechtitelů 27, 779 00 Olomouc, Czech Republic

E-mail: jan.strnad@upol.cz

We combined and compared gas chromatography (GC-FID) and photoacoustic detection (ETD-300) for measurement of ethylene production in plants. Both methods have been used in the past to measure ethylene production in plants, but a detailed comparison of these approaches and an attempt to merge them into a single approach, combining the advantages of both methods, have not yet been published. While GC has been proven to be very effective and requiring small amounts of sample gas, photoacoustic detection provides more sensitive measurements. Our approach involves measuring plant fresh mass, which is giving us a possibility to determine the endogenous levels 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 and/or whether the treatment actually affects ethylene production per se. Unlike typical methods, where plants are grown on treated media, we grow plants on untreated media and the treatment is applied at a precise time. This allows us to eliminate the effect of the treatment on germination. Another advantage is that the subsequent use of the combined approach on the same sample allows us to recognize machine errors that would otherwise be attributed to the biological variability. We are also able to save the samples for further hormonal analysis measurements. The method has been successfully tested for use with *Arabidopsis* and wheat plants treated with compounds known to affect ethylene production. Although the machines do not provide numerically identical values, the observed trends are identical.

The authors would like to acknowledge the support of the following grants: European Regional Development Fund-Project "SMART Plant Biotechnology for Sustainable Agriculture" (No. CZ.02.01.01/00/23_020/0008497), IGA-PrF_2026_014.

Anti-inflammatory and cytoprotective effects of 8-azapurines

Šomodiová K[1,3], Kordinová H[1], Gonzalez G[3], Kvasnica M[1], Mik V[2]

[1]Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences & Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

[2]Department of Experimental Biology, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

[3]Department of Neurology, University Hospital Olomouc and Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic.

E-mail: kristyna.somodiova01@upol.cz

Neurodegenerative diseases remain a group of disorders for which effective treatments are still missing. Their complex etiology is not yet fully understood, which complicates efforts to clarify the mechanisms underlying their onset and progression. These conditions hinder the development of novel therapeutics. Neuroinflammation represents one of the key mechanisms involved in neurodegeneration and has been intensively studied in recent years. It is well known that certain heavy metals can induce or exacerbate neuroinflammatory processes, thereby contributing to the development and progression of neurodegenerative diseases[1,2].

Previously optimized *in vitro* models of astrocytotoxicity and neuroinflammation were employed in this study. These models utilize the U-87 MG cell line differentiated into an astrocyte-like phenotype using all-*trans* retinoic acid. Copper was used as a cytotoxic inducer, while manganese served as a pro-inflammatory stimulus, resulting in two distinct experimental models suitable for testing potentially protective compounds. Based on the previous work of Kordinová et al., 2026, a novel series of compounds—8-azapurine derivatives—was synthesized and evaluated using these models. Several derivatives demonstrated significant astrocytoprotective and anti-inflammatory effects. The most promising compounds were able to reduce cellular and mitochondrial reactive oxygen species (ROS) production, decrease cell death, and reduce pro-inflammatory cytokine production.

Based on these findings, 8-azapurine derivatives represent a promising direction for the development of new therapeutic agents targeting neurodegenerative diseases.

The authors would like to acknowledge the support of the grants IGA_PrF_2026_014 and European Regional Development Fund-Project " Interdisciplinary Approaches for the Development and Application of New Materials in Medical Practice - New Omic Technologies " (No. CZ.02.01.01/00/23_021/0009224) co-funded by the European Union.

References

[1] **Giacoppo, S. et al., 2014.** Biological trace element research vol. 161 (2): 151-60.
[2] **Tarnacka B. et al., 2022.** International journal of molecular sciences vol. 22 (15): 7820.
[3] **Kordinová, H. et al. 2026**. European journal of medicinal chemistry vol. 301 (2026): 118217.

Novel disubstituted 1,2-diselenoles with stronger cytoprotective activity than Ebselen

Trofimenková D[1*], Bielczyński J[2*], Vítek D[3], Pigulski B[2], Múdrá M[1], Kryštof V[1], Szafert S[2], Voller J[1,3]

[1]*Department of Experimental Biology, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic*

[2]*Faculty of Chemistry, University of Wroclaw, F. Joliot-Curie 14, 50-383 Wrocław, Poland*

[3]*Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc, Hněvotínská 5, 77515 Olomouc, Czech Republic*

** - contributed equally*

E-mail: dominika.trofimenkova01@upol.cz

Ferroptosis is a newly characterized, regulated cell-death modality triggered by iron-driven oxidation of membrane lipids when glutathione-based defenses fail. Owing to these features, ferroptosis emerges as a promising therapeutic target in inflammatory diseases, cancer, aging, wound healing and retinal disorders such as age-related macular degeneration, diabetic retinopathy and ischemia-reperfusion-induced retinal degeneration[1].

Given the high susceptibility of 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[2].

Selenium-containing compounds present an attractive therapeutic approach, considering the role of selenium in cellular antioxidant mechanisms. This project investigates the protective effects of novel organoselenium compounds in retinal pigment epithelial cells (ARPE-19) exposed to structurally and mechanistically diverse ferroptosis inducers[3].

Cytoprotection was assessed via viability assays, including the resazurin and SRB assays. Direct radical quenching was assessed using biochemical evaluations such as the ORAC, FRAP, DPPH, ABTS and GPx activity assays.

Marked cytoprotection was observed already at concentrations of tens of nanomoles, as opposed to the clinical candidate ebselen, a GPx4 mimetic[4]. Their direct antioxidant capacity was stronger than that of ebselen, but did not exceed that of vitamin E analogue Trolox and cannot, therefore, fully explain the marked cytoprotective effect. Our research now focuses on the impact of these compounds on mitochondrial supercomplex stability under ferroptotic stress and employs lipid peroxidation probes to quantify lipid peroxidation and its suppression in cultured cells and liposomal systems.

The authors would like to acknowledge the support of the grants CZ-OPENSCREEN – LM2023052 from the Ministry of Education, Youth and Sports of the Czech Republic (MEYS), IGA_PrF_2025_ 011 from the Faculty of Science, Palacký University and IGA_LF_2025_021 from the Faculty of Medicine, Palacký University.

References

[1] **Dixon S.J. et al. 2012**. Cell 149 (5): 1060-1072.
[2 **Domènech E.B. and Marfany G. 2020**. Antioxidants 9 (4): 347.
[3] **Tapiero H. et al. 2003**. Biomedicine & Pharmacotherapy 57 (3-4): 134-144.
[4] **Sies H. and Masumoto H. 1996**. Advances in Pharmacology 38: 229-246.

Degradation of cyclin K and its impact on tumor cell proliferation

Vaško Š[1], Jorda R[1], Nencka R[2], Mik V[1], Vojáčková V[1], Břehová P[2], Kryštof V[1]

[1]Department of Experimental Biology, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

[2]Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Flemingovo nám. 2, 16000 Prague, Czech Republic

E-mail: simon.vasko01@upol.cz

Targeted protein degradation using molecular glues is an emerging therapeutic strategy, especially for modulating proteins that are difficult to inhibit with classical small molecules. One relevant example is cyclin K, which forms a functional complex with CDK12, a transcription-associated kinase that is important for proper expression of genes involved in the DNA damage response and genome stability. Previously described molecular glue degraders, including CR8, HQ461, and NCT02, have been shown to reshape the surface of CDK12 in a way that enables binding to the E3 ligase adaptor DDB1. This interaction recruits the CDK12–cyclin K complex to the CUL4–RBX1–DDB1 ubiquitin ligase machinery, leading to selective ubiquitination and proteasomal degradation of cyclin K. Mechanistically, cyclin K loss disrupts CDK12-dependent transcriptional programs, reduces expression of DNA repair genes, and produces antiproliferative effects in cancer cells. In this context, we have designed and synthesized a new series of compounds and are currently evaluating their effects on cyclin K degradation and CDK12-associated cellular functions.

The authors would like to acknowledge the support of the grant GACR 24-11114S.

CRE1-DWF5: Molecular Crosstalk Between Cytokinin Signaling and Brassinosteroid Biosynthesis

Kubalová I[1] and Zalabák D[1]

[1]Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences & Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

E-mail: david.zalabak@upol.cz

Cytokinins (CKs) and Brassinosteroids (BRs) are plant hormones that coordinate growth and development, yet their molecular crosstalk remains largely unexplored. Our findings uncover a novel regulatory axis linking CK signaling to BR biosynthesis via the direct interaction between the CK receptor CRE1 and DWF5, a key enzyme in BR biosynthesis. We observed that CK treatment increases levels of active BRs, whereas BR application reduces CRE1 abundance, revealing a dynamic two-way regulatory mechanism. Structural modeling further indicates that CRE1 phosphorylates DWF5, providing molecular evidence for a direct link between CK perception and BR synthesis. By elucidating how CK-BR crosstalk influences key developmental processes, our study aims to bridge fundamental hormone biology with translational applications, offering innovative strategies to enhance crop growth and yield while addressing the sustainable demands of agriculture.

The authors would like to acknowledge support from grant 26-21113S *from the Czech Science Foundation and JG_2026_025 from the Palacký University in Olomouc.*

Integrative analysis of the barley mutant chlorina f2^{f2} reveals coordinated changes in photosynthesis, hormone homeostasis, and stress responses

Zemánková N[1], Chamrád I[1], Petřík I[1], Široká J[1], Ament A[1], Plačková L[1], Lenobel R[1], Novák O[1]

[1]Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences & Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

E-mail: natalie.zemankova@upol.cz

Barley (Hordeum vulgare) is a major cereal crop and a key model for studying chloroplast structure and function, supported by the availability of diverse mutant lines. Among these, chlorina f2^{f2}, characterized by a severe reduction or absence of chlorophyll b, has been extensively used to investigate the role of chlorophyll in photosynthesis and thylakoid organization. However, a systems-level understanding of its molecular and physiological phenotype remains lacking. Here, we present an integrative analysis of the chlorina f2^{f2} mutant combining whole-tissue and subcellular proteomics, detailed hormone-level determination, and selected physiological assays. Whole leaf tissue from seven-day-old wild-type barley (cv. Morex) and chlorina f2^{f2} was subjected to comprehensive proteomic profiling and complemented by analysis of isolated chloroplasts and thylakoid membranes. In good agreement with published results, this approach revealed a pronounced and coordinated downregulation of proteins involved in photosynthesis, chlorophyll biosynthesis, and thylakoid organization. Furthermore, a broader rewiring of pathways linked to hormone metabolism and stress responses was observed, highlighted by the downregulation of proteins involved in the biosynthesis of auxins, gibberellins, and jasmonates, alongside reduced abundance of proteins associated with oxidative stress-related processes, including response to oxidative stress, response to toxic substances, and hydrogen peroxide catabolism. To further dissect these changes, we performed targeted phytohormone profiling focusing on jasmonates, auxins, and cytokinins, revealing substantial shifts in key regulators of growth and stress signaling. Consistent with proteomic signatures indicating altered oxidative stress pathways, stress-response experiments demonstrated changes in reactive oxygen species (ROS) accumulation in the mutant background. Complementary pigment analyses further revealed altered levels of chlorophyll a, chlorophyll b, carotenoids, and anthocyanins, reinforcing the extent of physiological perturbations. Collectively, our findings demonstrate that chlorina f2^{f2} extends beyond a simple model of chlorophyll b deficiency and instead represents a complex system integrating perturbations in photosynthesis, hormonal regulation, and stress signaling. From this perspective, it provides a valuable resource for future studies aimed at dissecting the interplay between pigment biosynthesis, photosynthetic efficiency, and plant stress responses.

The authors would like to acknowledge the support of the grant IGA_PrF_2026_014 provided by Internal grant agency of Palacký University Olomouc.

Natural abundance of 4-Cl-IAA and its metabolites in plants

Žukauskaitė A[1], Drbohlavová H[1], Bieleszová K[1], Najdeková N[1], Novák O[2], Hladík P[2], Pěnčík A[2]

[1]Department of Chemical Biology, Faculty of Science, Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

[2]Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences & Palacký University Olomouc, Šlechtitelů 27, 77900, Olomouc, Czech Republic.

E-mail: asta.zukauskaite@upol.cz

Auxin is a key regulator of plant growth and development, with indole-3-acetic acid (IAA) being the most extensively studied natural auxin. IAA homeostasis is maintained through a dynamic and well characterized network of biosynthesis, conjugation, degradation, and transport[1].

Another naturally occurring auxin, 4-chloroindole-3-acetic acid (4-Cl-IAA) possesses strong auxinic activity, in bioassays often outperforming IAA[2]. Despite its physiological potency, species known to synthesize 4-Cl-IAA appear to be restricted to the phylogenetic clades of the Fabeae and Trifoleae in the Fabaceae family, which includes pea (*Pisum sativum* L.), where it plays essential roles in fruit and seed development[3].

Current evidence suggests that 4-Cl-IAA biosynthesis in pea proceeds from 4-chloro-tryptophan (4-Cl-Trp) via 4-chloro-indole-3-pyruvic acid (4-Cl-IPyA), analogous to the Trp-dependent IPyA pathway established for IAA[4]. However, beyond this proposed biosynthetic route, virtually nothing is known about the downstream metabolism of 4-Cl-IAA and it remains unclear whether it could be metabolized by the same enzyme families that act on IAA or whether it follows distinct, pea-specific pathways.

Herein, we report the synthesis of putative 4-Cl-IAA metabolites, optimization of their purification and analytical methods, and subsequent quantitative analysis in selected plant species.

The authors would like to acknowledge financial support from the project TowArds Next GENeration Crops (TANGENC) (reg. no. CZ.02.01.01/00/22_008/0004581), funded by the ERDF Programme Johannes Amos Comenius, and from the Internal Grant Agency of Palacký University Olomouc (IGA_PrF_2026_013, and IGA_PrF_2026_014).

References

[1] **Hayashi K. et al. 2021**. Nature Communications 12: 6752.
[2] **Karcz W. et al. 2002**. Journal of Experimental Botany 53(371): 1089–1098.
[3] **Jayasinghege C.P.A. et al. 2019**. Journal of Experimental Botany 70(4): 1239–1253.
[4] **Tivendale N.D. et al. 2012**. Plant Physiology 159(3): 1055–1063.