

# 12<sup>th</sup> PSEPB Conference

*plantæ vita sunt*

September  
9-12, 2025

Faculty of Biology  
University of Warsaw  
Miecznikowa 1, Warsaw



Polish Society of Experimental  
Plant Biology



UNIVERSITY  
OF WARSAW

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# 12<sup>th</sup> PSEPB Conference

## Welcome to the 12th PSEPB 2025 Conference

The Polish Society of Experimental Plant Biology warmly welcomes experimental plant biologists to our 12th Biennial Conference. Every other year, our meetings bring together plant biologists from across Poland and around the world to share cutting-edge research and foster collaboration.

This year's congress, themed *Plantae vita sunt* (Plants are life), emphasizes the fundamental importance of plants to our planet and their crucial role in addressing contemporary global challenges.

This congress features sessions covering a broad spectrum of plant biology research areas. We're excited to welcome a fantastic group of speakers from Europe and beyond who are eager to share their latest discoveries. The event is dedicated to showcasing groundbreaking plant biology research from around the world, with a special opportunity to elevate the visibility of innovative work being conducted in Poland.

# Program

Tuesday, September 9, 2025

**13:30 REGISTRATION**

**15:30–15:45 OPENING**

**15:45–16:30 OPENING LECTURE**

**Paweł Łuków (University of Warsaw)**

*The Moral Value of Basic Research or the Significance of the Unspectaculars*

**16:30–17:30 PSEPB AWARD CEREMONY**

*Awards presented by a member of the Prize Committee*

**Magdalena Arasimowicz-Jelonek (Adam Mickiewicz University, Poznań)**

**17:30–20:00 WELCOME RECEPTION**

# Wednesday, September 10, 2025

## SESSION 1: DNA AND RNA METABOLISM, EPIGENETICS AND GENOMICS

Session Organizers: Rafał Archacki, Jakub Dolata

### 08:30 – 09:05 INTERNATIONAL KEYNOTE SPEAKER

**Rémy Merret (IBMP - CNRS - Université de Strasbourg)**

*Interplay between mRNA decay and translation in plants*

### 09:05 – 09:40 NATIONAL KEYNOTE SPEAKER

**Piotr Ziółkowski (Adam Mickiewicz University, Poznań)**

*How to make sixty crossovers out of eight: Boosting meiotic recombination in plants*

### 09:40 – 10:30 SELECTED ORAL PRESENTATIONS (5 × 10 MINUTES)

#### 09:40 – 09:50 Wojciech Strzałka

*Biological activity of Arabidopsis flap endonuclease 1 (FEN1) is modulated by nuclear factors that inhibit its aggregation*

#### 09:50 – 10:00 Tomasz Bieluszewski

*Mechanisms for overcoming Polycomb silencing in plants*

#### 10:00 – 10:10 Katarzyna Kapela

*Catalytic activity of the BRM ATPase, and BDH1/2 subunits are necessary for the functions of SWI/SNF complex in Arabidopsis*

#### 10:10 – 10:20 Tomasz Sarnowski

*Unlocking cancer mechanisms: The role of Arabidopsis thaliana in understanding regulatory impairments in cancer*

#### 10:20 – 10:30 Agata Daszkowska-Golec

*Seeing is believing: tissue-resolved insights from spatial transcriptomics into ABA signaling during barley seed germination*

### 10:30 – 11:00 COFFEE BREAK

## SESSION 2: PROTEOMICS, METABOLOMICS AND PHENOMICS

Session Organizers: Paweł Sowiński, Tomasz Pawłowski

### 11:00 – 11:35 INTERNATIONAL KEYNOTE SPEAKER

**James Schnable (University of Nebraska-Lincoln)**

*What We Can See, We Can Change: Linking Plant Form to Gene Function*

### 11:35 – 12:10 NATIONAL KEYNOTE SPEAKER

**Robert Nawrot (Adam Mickiewicz University, Poznań)**

*Pathogenesis-related proteins from *Chelidonium majus* L. latex as components of plant defense system*

### 12:10 – 13:00 SELECTED ORAL PRESENTATIONS (5 × 10 MINUTES)

#### 12:10 – 12:20 Tomasz Pawłowski

*Molecular Mechanisms of European Beech Adaptation to Environmental Conditions in the Context of Seed Germination*

#### 12:20 – 12:30 Brian Wakimwayi Koboyi

*Analysis of proteomic changes in rye (*Secale cereale* L.) seedlings under phosphate deficiency using LC-MS/MS*

#### 12:30 – 12:40 Andżelika Drozda

*NO-mediated Regulation of Seed Dormancy and Germination in European Beech through Protein Post-Translational Modifications*

- 12:40 – 12:50 Anna Ihnatowicz**  
*Integrating Mutant Approaches, Natural Variation, and Multi-Omics to Uncover the Role of Coumarins in Arabidopsis Stress Responses*
- 12:50 – 13:00 Karolina Skorupinska-Tudek**  
*Polyprenols, dolichols and their derivatives as biosynthetic intermediated, cofactors and modulators of biological membrane properties*

**13:00 – 14:30 LUNCH**

### **SESSION 3: APPLICATION OF PLANT BIOTECHNOLOGY FOR SUSTAINABLE AGRICULTURE**

*Session Organizers: Ewa Łojkowska, Anna Ihnatowicz*

- 14:30 – 15:05 INTERNATIONAL KEYNOTE SPEAKER**  
**Seth J. Davis (University of York)**  
*Linking biological clock genes with developmental traits related to yield*
- 15:05 – 15:40 NATIONAL KEYNOTE SPEAKER**  
**Rafał Barański (University of Agriculture in Krakow)**  
*What we have learned from carrot genome editing: from basic research to challenges for future application*
- 15:40 – 16:30 SELECTED ORAL PRESENTATIONS (4 × 10 MINUTES)**
- 15:40 – 15:50 Agnieszka Zienkiewicz**  
*Targeting lipid degradation to enhance TAG accumulation in Arabidopsis leaves*
- 15:50 – 16:00 Sylwia Klińska**  
*Effect of Temperature Stress on Phenotype of Arabidopsis thaliana PDAT1 Mutant Lines*
- 16:00 – 16:10 Danuta Wójcik**  
*Studies on the increased tolerance of apple polyploids to biotic stresses*
- 16:10 – 16:20 Magdalena Cieplak**  
*BSA-seq approach identifies a genomic region associated with male sterility in sweet pepper*
- 16:30 – 18:00 POSTER SESSION 1 (ODD-NUMBERED POSTERS) + NETWORKING EVENT**

# Thursday, September 11, 2025

## SESSION 4: BIOLOGY OF CHLOROPLASTS AND PLANT MITOCHONDRIA

Session Organizers: Bożena Szal, Beata Myśliwa-Kurdziel

### 08:30 – 09:05 INTERNATIONAL KEYNOTE SPEAKER

**Wojciech Nawrocki (French National Centre for Scientific Research - CNRS)**

*Light harvesting regulation and photodamage interplay in Chlamydomonas reinhardtii*

### 09:05 – 09:40 NATIONAL KEYNOTE SPEAKER

**Małgorzata Kwaśniak-Owczarek (University of Wrocław)**

*The secrets of ribosomes and post-transcriptional events in plant mitochondria*

### 09:40 – 10:30 SELECTED ORAL PRESENTATIONS (5 × 10 MINUTES)

#### 09:40 – 09:50 Alicja Bukat

*Ultrastructural analysis of prolamellar body formation in oat leaves*

#### 09:50 – 10:00 Justyna Łabuz

*A large-scale study of chloroplast movements in wild plant species*

#### 10:00 – 10:10 Małgorzata Adamiec

*Chloroplast intramembrane proteases: important factors for photosynthetic efficiency in Arabidopsis thaliana*

#### 10:10 – 10:20 Zuzanna Jakubowska

*Photoprotective role of PsbS and zeaxanthin in seedlings of Arabidopsis thaliana npq1 and npq4 mutants*

#### 10:20 – 10:30 Katsiaryna Kryzheuskaya

*The mitochondrial NDA2 dehydrogenase as a key regulator of Arabidopsis responses to abiotic stresses*

### 10:30 – 11:00 COFFEE BREAK

## SESSION 5: ADVANCES IN PLANT STRUCTURE AND DEVELOPMENT

Session Organizers: Edyta Gola, Alicja Dołzbłasz

### 11:00 – 11:35 INTERNATIONAL KEYNOTE SPEAKER

**Thomas Laux (Faculty of Biology, University of Freiburg, Germany)**

*Making a pattern: lessons from plant embryogenesis*

### 11:35 – 12:10 NATIONAL KEYNOTE SPEAKER

**Maciej Adamowski (Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk)**

*The endomembrane system in plant developmental patterning*

### 12:10 – 13:00 SELECTED ORAL PRESENTATIONS (5 × 10 MINUTES)

#### 12:10 – 12:20 Barbara Wójcikowska

*Uncovering genes under MONOPTEROS regulation during the embryogenic transition in Arabidopsis thaliana*

#### 12:20 – 12:30 Małgorzata Lichocka

*Annexin 5 is a novel regulator of ribosomal RNA reserves accumulation during pollen development in Arabidopsis thaliana.*

#### 12:30 – 12:40 Wiktoria Wodniok

*In search of relationships between hypocotyl growth, mechanics and hemicelluloses of primary cell walls*

#### 12:40 – 12:50 Jędrzej Dobrogojski

*Charge of the plant extracellular matrix and its role in calcium homeostasis*



**12:50 – 13:00 Aleksandra Liszka**  
*Molecular insights into VND-Mediated cell wall formation in conifers*

**13:00 – 14:30 LUNCH**

**SESSION 6: PLANTS AND ABIOTIC STRESSES**

*Session Organizers: Arkadiusz Kosmala, Magdalena Arasimowicz-Jelonek*

**14:30 – 15:05 INTERNATIONAL KEYNOTE SPEAKER**  
**Tibor Janda (Department of Plant Physiology and Metabolomics, Agricultural Institute, HUN-REN Centre for Agricultural Research, Hungary)**  
*Light signal transduction from shoot to root at low temperatures in cereals*

**15:05 – 15:40 NATIONAL KEYNOTE SPEAKER**  
**Grażyna Dobrowolska (Institute of Biochemistry and Biophysics, Polish Academy of Sciences)**  
*Abiotic Stress Signaling – Follow the SnRK2 Pathways*

**15:40 – 16:30 SELECTED ORAL PRESENTATIONS (4 × 10 MINUTES)**

**15:40 – 15:50 Magdalena Korek**  
*The cost of survival: Barley HvD53A mutation boosts drought tolerance at the expense of Photosynthesis*

**15:50 – 16:00 Dorota Sołtys-Kalina**  
*DNA methylation signatures of drought stress priming in potato cultivars*

**16:00 – 16:10 Katarzyna Leja**  
*TATOOINE: A novel chloroplast-localized protein involved in plant drought response*

**16:10 – 16:20 Alicja Dobek**  
*Novel O-demethylation activity leads to esculetin biosynthesis and impacts stress response*

**16:30 – 18:00 POSTER SESSION 2 (EVEN-NUMBERED POSTERS) + NETWORKING EVENT**



# Friday, September 12, 2025

## SESSION 7: PLANT BIOTIC INTERACTIONS

Session Organizers: Aleksandra Obrępańska-Stęplowska, Magdalena Krzymowska

### 08:00 – 08:35 INTERNATIONAL KEYNOTE SPEAKER

**Alberto Carbonell** (Instituto de Biología Molecular y Celular de Plantas, Valencia, Spain)

*Small RNAs and Argonautes in Plant-Virus Interactions: Mechanistic Insights and Biotechnological Applications for Precision Crop Protection*

### 08:35 – 09:10 NATIONAL KEYNOTE SPEAKER

**Michał Jasiński** (Institute of Bioorganic Chemistry, Polish Academy of Sciences)

*Cytokinins in and out - how ABC driven transport shapes Medicago root morphology upon nitrogen deficiency and interactions with Rhizobia*

### 09:10 – 10:00 SELECTED ORAL PRESENTATIONS (5 × 10 MINUTES)

#### 09:10 – 09:20 Juan Ochoa

*Type three effector HopBF1 of Pseudomonas syringae induces systemic micronecroses in Nicotiana benthamiana*

#### 09:20 – 09:30 Ton Timmers

*Nod factor perception and signal transduction during endosymbiotic interactions of Medicago*

#### 09:30 – 09:40 Sanjana Sarode

*Aphid-Mediated Virus Infection Alters Volatile Organic Compound Emissions in the Pepper*

#### 09:40 – 09:50 Justyna Lalak-Kańczugowska

*C-terminal part of phytochelatase synthase supports its function in pathogen-triggered indole glucosinolate metabolism in Brassicaceae*

#### 09:50 – 10:00 Thibault Barrit

*Using GWAS and the Arabidopsis–Dickeya pathosystem to elucidate links between iron nutrition, coumarins, and plant defense*

### 10:00 – 10:15 COFFEE BREAK

## SESSION 8: SHORT- AND LONG-DISTANCE COMMUNICATION IN PLANTS

Session Organizers: Ewa Kurczyńska, Agnieszka Zienkiewicz

### 10:15 – 10:50 INTERNATIONAL KEYNOTE SPEAKER

**Yoselin Benitez-Alfonso** (Interdisciplinary Plant Sciences, University of Leeds, United Kingdom)

*Plasmodesmata cell walls: the mechanical and structural properties that control communication*

### 10:50 – 11:25 NATIONAL KEYNOTE SPEAKER

**Urszula Krasuska** (Department of Plant Physiology, Institute of Biology, Warsaw University of Life Sciences)

*Small but powerful - plant gasotransmitters in (mode of) action*

### 11:25 – 12:15 SELECTED ORAL PRESENTATIONS (5 × 10 MINUTES)

#### 11:25 – 11:35 Wiktoria Parzych

*Structural Pathways and Functional Implications of Cell-to-Cell Communication in Arabidopsis thaliana Male and Female Germ Units*

#### 11:35 – 11:45 Małgorzata Gutkowska

*Dolichol biosynthesis mutant lew1 affects pollen development and plant fertility in Arabidopsis*

- 11:45 – 11:55 Maciej Nowak**  
*Role of MOTHER OF FT AND TFL1 in Seed Development and Germination in Medicago truncatula*
- 11:55 – 12:05 Brygida Świeżawska-Boniecka**  
*When hormones meet signal molecules - understanding strigolactone and cNMP crosstalk in plants*
- 12:05 – 12:15 Oskar Siemianowski**  
*How do plants manage their microelements? Zinc Translocation from Zn-Sufficient to Zn Deficient Roots as an Adaptation to Heterogeneous Zn Availability*
- 12:15 – 13:00 LUNCH**
- SESSION 9: EARLY LAND PLANT BIOLOGY**  
*Session Organizers: Zofia Szweykowska-Kulińska, Agnieszka Hanaka*
- 13:00 – 13:35 INTERNATIONAL KEYNOTE SPEAKER**  
**Liam Dolan (Gregor Mendel Institute, Vienna, Austria)**  
*De novo Development of Plant Meristems*
- 13:35 – 14:10 NATIONAL KEYNOTE SPEAKER**  
**Jakub Sawicki (Department of Botany and Evolutionary Ecology, University of Warmia and Mazury, Olsztyn)**  
*Fast-evolving Liverworts: A Genomic Perspective from Apoppelia endiviifolia (Pelliales, Jungermaniopsida)"*
- 14:10 – 15:00 SELECTED ORAL PRESENTATIONS (5 × 10 MINUTES)**
- 14:10 – 14:20 Jan Żeruń**  
*Changes of biochemical composition in Chlamydomonas reinhardtii treated with brassinolide*
- 14:20 – 14:30 Izabela Sierocka**  
*MpSPL3 gene is indispensable for generative organs development in Marchantia polymorpha*
- 14:30 – 14:40 Bharti Aggarwal**  
*Study on liverwort-specific miRNAs – MpmiR11796 and MpmiR11887 involved in the sexual organ development of Marchantia polymorpha*
- 14:40 – 14:50 Halina Pietrykowska**  
*MpmiR11889 controls sperm cell formation and boosts reproductive efficiency in Marchantia polymorpha*
- 14:50 – 15:00 Jędrzej Szymański**  
*Using AI to Predict Gene Regulation and Guide Experiments in Plants*
- 15:00 – 15:30 CLOSING CEREMONY AND POSTER AWARD CEREMONY**

# Oral presentation abstracts

# Interplay between mRNA decay and translation in plants

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Throughout its lifetime, messenger RNA (mRNA) exists in a dynamic balance between translation, storage, and decay. The spatiotemporal regulation of this equilibrium is essential for fine-tuning the transcriptome in response to developmental and environmental cues in plants. As an example, in *Arabidopsis*, disruption of mRNA decay due to the loss of key regulatory factors leads to post-embryonic lethality, severe growth defects, or impaired stress responses. Despite its critical role in gene expression regulation, our understanding of the interplay between mRNA translation, storage, and decay remains limited in multicellular organisms. In this presentation, I will share recent findings from my group on how translation and mRNA decay interact in plants, highlighting their importance in plant development and responses to stress. I will also present preliminary data suggesting that codon composition may influence this interplay in *Arabidopsis*.

**DNA and RNA metabolism,  
epigenetics and genomics**

**Keynote lecture**

**Authors:**

**Rémy Merret**

*Institut de biologie moléculaire des  
plantes, CNRS, Université de Strasbourg*

# How to make sixty crossovers out of eight: Boosting meiotic recombination in plants

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In sexually reproducing organisms, crossover formation during meiosis is tightly controlled. In *Arabidopsis thaliana*, only about eight crossovers occur per meiosis, mainly through the class I pathway, which depends on meiosis-specific ZMM proteins. A second, class II pathway is typically suppressed by anti-recombination helicases such as FANCM and RECQ4. To enhance class II crossover activity, we disrupted genes that limit recombination. Unexpectedly, combining mutations in RECQ4 and ZIP4 (a core ZMM protein) led to a nearly twofold increase in crossovers compared to *recq4* mutants alone – without increasing DNA double-strand breaks. This suggests that ZMM proteins not only promote class I crossovers but also limit class II crossovers by protecting recombination intermediates from cleavage by structure-specific endonucleases. Additional increases were achieved by mutating MSH2, a mismatch repair gene that suppresses recombination independently of ZMMs. In higher-order mutants lacking FANCM, RECQ4, ZIP4, and MSH2, crossover numbers rose over ninefold relative to wild type. These results demonstrate that crossover frequency is more flexible than previously believed. By removing multiple layers of suppression, meiotic recombination can be significantly boosted. This has important implications for plant breeding and genetic mapping by expanding genetic diversity beyond natural limits.

## *additional info*

This research was supported by NCN grants no. 2020/39/I/NZ2/02464 and 2024/54/A/NZ2/00266 awarded to P.A.Z.

**DNA and RNA metabolism, epigenetics and genomics**

## **Keynote lecture**

### **Authors:**

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**Alexandre Pelé**

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**Maja Szymańska-Lejman**

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# Biological activity of Arabidopsis flap endonuclease 1 (FEN1) is modulated by nuclear factors that inhibit its aggregation

Flap endonuclease 1 (FEN1) is a eukaryotic nuclear enzyme involved in the maturation of Okazaki fragments during DNA replication. Our recent biochemical and biophysical studies of FEN1 from the model plant Arabidopsis (AtFEN1) revealed that this protein is prone to aggregation. Based on this observation, the aim of the present study was to elucidate the causes of this phenomenon. Studying effects of heparin sodium and sodium chloride on AtFEN1 aggregation we found that both agents modulated AtFEN1 aggregation. Achieving the same level of aggregation inhibition required a five-orders-of-magnitude higher concentration of sodium chloride compared to heparin. To identify potential nuclear factors that may modulate the biological activity of AtFEN1 *in vivo*, we examined various types of DNA. Double-stranded DNA (dsDNA), similarly to the double-flap DNA (dfDNA) - the natural substrate of AtFEN1, inhibited its aggregation. In contrast, the protective effect of single-stranded DNA (ssDNA) was significantly weaker. Moreover, dfDNA was shown to have a positive effect on maintaining the biological activity of AtFEN1. Finally, we demonstrated that proliferating cell nuclear antigen (PCNA), a known interaction partner of AtFEN1, also prevented its aggregation. However, this effect was observed only in the presence of the putative PCNA-interacting protein (PIP)-box sequence in AtFEN1.

## *additional info*

This project was supported by the National Science Center, Poland, under grant UMO-2019/33/B/NZ3/01568 to W.S.

**DNA and RNA metabolism, epigenetics and genomics**

## **Oral presentation**

### **Authors:**

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# Mechanisms for overcoming Polycomb silencing in plants

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Polycomb silencing creates a barrier to the activation of genes outside of their proper expression domain. Activation of Polycomb-silenced genes, initiated by DNA sequence-specific transcription factors requires a variety of co-activators and chromatin regulators conserved in plants and animals. Which of these factors are sufficient to start the reprogramming process remains largely unknown. We used a synthetic biology approach to screen a representative set of plant activators with the aim of identifying mechanisms of Polycomb target activation genome-wide. In doing so, we developed a promising new method for redirecting plant development.

**DNA and RNA metabolism,  
epigenetics and genomics**

**Oral presentation**

**Authors:**

**Tomasz Bieluszewski**

*Adam Mickiewicz University  
University of Pennsylvania*

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*University of Pennsylvania*



# Catalytic activity of the BRM ATPase, and BDH1/2 subunits are necessary for the functions of SWI/SNF complex in Arabidopsis

SWI/SNF chromatin remodelers are evolutionarily conserved multiprotein complexes that use the energy of ATP hydrolysis to change chromatin structure. In Arabidopsis, SWI/SNF remodelers can be categorized into three subtypes, each containing either BRAHMA (BRM), SPLAYED (SYD) or MINUSCULE1/2 (MINU1/2) ATPase, along with several accessory subunits. BDH1 and 2 are redundant subunits present in all three SWI/SNF classes, and have been proposed to influence SWI/SNF activity. Here, we investigate how catalytic activity of the BRM ATPase, and BDH1/2 subunits contribute to the overall SWI/SNF complex functionality. First, we generated Arabidopsis lines expressing point mutations in the catalytic domain of BRM. We show that these mutations cause developmental and gene expression defects that are less severe from those present in brm-1 null mutant. Furthermore, we found that bdh1/2 mutation strongly enhanced the effects of the BRM point mutation, suggesting important role of BDH1/2 in the regulation of the SWI/SNF catalytic activity. To gain deeper insight of how the studied mutations affect catalytic activity and association of SWI/SNF with chromatin, we performed ATPase assays and salt fractionation analyses. Collectively, our results reveal activity-dependent and independent functions of the SWI/SNF complex in Arabidopsis.

## additional info

This research was funded by NATIONAL SCIENCE CENTRE POLAND, grants number 2017/26/E/NZ2/00899 and 2023/49/N/NZ2/03263.

**DNA and RNA metabolism, epigenetics and genomics**

**Oral presentation**

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# Unlocking cancer mechanisms: The role of *Arabidopsis thaliana* in understanding regulatory impairments in cancer

In 2019, cancer accounted for about 25% of male and 23% of female deaths in Poland, underscoring a major public health concern. Understanding tumor formation mechanisms is essential, and *Arabidopsis thaliana* serves as a valuable model for studying regulatory processes, including epigenetic control of gene expression. Our research employs both *Arabidopsis* and human cancer cell line models to explore disrupted regulatory mechanisms contributing to carcinogenesis. Malignant tumors often show defects in critical regulatory pathways, particularly in gene expression control. Approximately 25% of cancers involve mutations in genes coding for subunits of the SWI/SNF chromatin remodeling complexes (CRCs), which play a vital role in modulating gene expression. Our study of *Arabidopsis* lines with mutations in SWI/SNF CRCs genes has revealed interdependencies between ATP-dependent chromatin remodeling and regulatory pathways involving metabolism and plasma membrane receptors. We found that the human counterparts of these factors exhibit similar regulatory mechanisms. Inactivation of some SWI/SNF CRCs subclasses may cause formation of pathological/non-canonical SWI/SNF CRCs variants and affect the distribution of other SWI/SNF subclasses. Collectively, our results suggest a universal paradigm for transcriptional control across plant and animal kingdoms. Our findings propose novel molecular targets for innovative cancer treatments, particularly through the concept of synthetic lethality.

*additional info*

Narodowe Centrum Nauki: 2018/30/M/NZ1/00180.

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**DNA and RNA metabolism,  
epigenetics and genomics**

**Oral presentation**

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# Seeing is believing: tissue-resolved insights from spatial transcriptomics into ABA signaling during barley seed germination

Understanding the spatial dimension of hormone action is crucial for decoding regulatory networks during seed germination. Here, we integrate Visium Spatial Transcriptomics (10x Genomics) with bulk transcriptomics and metabolomic profiling to generate the first tissue-resolved, multi-omic picture of abscisic acid (ABA)-influenced germination in barley (*Hordeum vulgare*) embryos. Barley embryos treated with ABA or mock solution were cryosectioned and spatial barcoding captured high-resolution expression profiles for over 2,000 ABA-responsive genes. Notably, 49 of these genes exhibited strict tissue restriction, with the coleoptile alone accounting for 30, including 14 unique regulators - positioning it as the principal integration hub for stress signals. By overlaying bulk transcriptomic and metabolomic data, we observed a three-fold rise in ABA and carotenoid precursors that paralleled activation of the PP2C–SnRK2–ABF signaling module and coordinated repression of gibberellin, jasmonate and auxin pathways. Distinct regional signatures using spatial transcriptomics further delineated roles in cell-wall remodeling, nutrient mobilization, redox homeostasis and developmental arrest under ABA. Our results demonstrate that spatial transcriptomics fills a critical gap left by bulk RNA-seq and metabolomics alone, revealing fine-scale regulatory landscapes that govern seed physiology.

## additional info

This work was supported by the National Science Center, Poland project SONATA BIS10 '(QUEST) Quest for climate-smart barley—the multilayered genomic study of CBC function in ABA signaling' (2020/38/E/NZ9/00346).

**DNA and RNA metabolism, epigenetics and genomics**

**Oral presentation**

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# What We Can See, We Can Change: Linking Plant Form to Gene Function

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The vast majority of annotated gene models in plant genomes, including well characterized genetic models, are not linked to phenotypic outcomes via experimental evidence. The effort required to characterize the function of a given gene has not experienced the same rapid productivity gains as genomic sequencing and high throughput plant phenotyping. This presentation discusses how rich genomic and phenomic data can improve the efficiency and impact of gene characterization. Modern high throughput phenotyping makes it feasible to collect feature-rich time-series phenotypic datasets. By identifying specific phenotypic features associated with overall crop performance, explainable AI approaches can help prioritize traits for in depth genetic characterization. A combination of higher density genetic markers and transcriptome-wide association accelerate the identification of candidate genes contributing to traits of interest. Models trained on sets of genes which have already been linked to phenotypes may reduce the greatest risk of reverse genetics strategies: genes with no apparent loss of function phenotype. Together, these can both advance fundamental research into the function of the large proportion of plant genes not linked to function, and improve translatability between fundamental plant genetic investigation and our ability to design and deploy crops that meet the challenges of tomorrow's environments.

## *additional info*

This work was supported by the US National Science Foundation, the US Department of Energy, and the Nebraska Corn Growers.

**Proteomics, metabolomics and phenomics**

**Keynote lecture**

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# Pathogenesis-related proteins from *Chelidonium majus* L. latex as components of plant defense system

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Plants have developed intricate defense mechanisms against pathogen infections, herbivores and biotic and abiotic factors. The important components of this system comprise plant pathogenesis-related proteins (PR). Greater Celandine (*Chelidonium majus* L.) is a model medicinal plant from the family Papaveraceae, with a range of biological activities, like antiviral, antimicrobial, proapoptotic or cytotoxic. Although the extracts from these plants have long been used in traditional folk medicine, the exact molecular mechanism of their action still remains unknown. After cutting the plant, it exudes an orange-yellow milky juice (latex) rich in many low-molecular-weight compounds (like isoquinoline alkaloids, flavonoids, phenolic acids) as well as proteins. Two proteins are of our particular interest - major latex protein (MLP) and glycine-rich protein (GRP). *C. majus* MLP and GRP proteins are possible factors of latex antiviral activity, and this activity may be enhanced by other latex components. *C. majus* latex contains also other members of PR family, like recently discovered antimicrobial CmAMP1. Proteins can facilitate the transport of low-molecular-weight compounds into the cell or even play a role of their transporters. The aim of the project is to elucidate the structure, function and molecular mechanism of *Chelidonium majus* defense-related proteins and their antiviral and anticancer activities.

**Proteomics, metabolomics and phenomics**

**Keynote lecture**

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# Molecular Mechanisms of European Beech Adaptation to Environmental Conditions in the Context of Seed Germination

Seed dormancy is a key adaptation that synchronizes germination with favourable environmental conditions. However, the integration of dormancy-regulating mechanisms under natural conditions remains poorly understood. This study combines data from ecology, transcriptomics, proteomics, and epigenetics to investigate seed germination processes. Seeds from three European beech populations differing in dormancy depth were analyzed. Dormant, cold-stratified (non-dormant), and germinated seeds were used to examine gene expression at both the mRNA and protein levels, including post-translational modifications. LC-MS/MS and NGS methods were applied. The analysis revealed significant differences in protein and RNA expression related to both germination and population origin. Functional annotation showed that the affected proteins and transcripts are involved in hormone signalling, methionine biosynthesis, and gene expression regulation. Findings suggest that beech populations exhibit distinct adaptive strategies for seed germination at both the transcriptomic and proteomic levels. Germination had a stronger impact on gene expression than population differences, indicating a robust physiological response to environmental cues. These results support the development of a model explaining how beech seeds adapt their germination behaviour to varying environmental conditions.

## *additional info*

This research was supported by the National Science Centre, Poland (Project 2019/33/B/NZ9/02660).

**Proteomics, metabolomics and phenomics**

**Oral presentation**

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# Analysis of proteomic changes in rye (*Secale cereale* L.) seedlings under phosphate deficiency using LC-MS/MS

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Phosphorus is a vital macronutrient for plant growth, and its deficiency evokes an array of changes that alter physiological and molecular processes as an adaptive mechanism to promote efficient phosphorus uptake and utilization. We aim to understand the mechanism of phosphorus tolerance in the rye (*Secale cereale* L.) shoots and roots. Total protein extraction using an (Sodium Dodecyl Sulphate) SDS-containing extraction buffer + phenol clean-up step was carried out on the shoots and roots of rye (*Secale cereale* L.) inbred lines seedlings grown in a hydroponic system of 0.2 mM KH<sub>2</sub>PO<sub>4</sub> in control (+P) conditions and 0.01 mM KH<sub>2</sub>PO<sub>4</sub> (with additional 0.19 mM KCl in phosphorus-deficient conditions (-P)). The isolated proteins were further purified using chloroform-methanol precipitation for compatibility with LC-MS/MS with Tandem Mass Tag labelling (TMT) analysis. A significant number of differentially expressed proteins were identified in both the *Secale cereale* and *Triticum aestivum* databases. Particularly those involved in oxidative stress mitigation, stress response, phosphate transport and metabolic adaptation. The Principal Component Analysis (PCA) revealed that the genotype explains the variation between the shoot and root samples. Additionally, the PCA showed that the samples of the same genotype and condition group together, indicating a successful experimental stage.

## additional info

This study was funded by grant No. 2020/37/B/NZ9/00738 from the National Science Centre, Poland.

**Proteomics, metabolomics and phenomics**

**Oral presentation**

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# NO-mediated Regulation of Seed Dormancy and Germination in European Beech through Protein Post-Translational Modifications

Seed dormancy is a key adaptive trait that synchronizes germination with favorable environmental conditions. Nitric oxide (NO)-mediated post-translational modifications (PTMs), specifically S-nitrosylation and tyrosine nitration, act as redox-regulated modulators of protein function during dormancy transitions. Here, we investigated NO-dependent PTMs in European beech (*Fagus sylvatica* L.) seeds from three natural populations differing in dormancy depth. Seeds at three distinct developmental stages – dry dormant, cold-stratified, and germinated – were analyzed using a range of analytical techniques, including fluorescence-based NO quantification, Western blotting, and LC-MS/MS proteomics. Our findings revealed unique patterns of protein S-nitrosylation and nitration that were specific to both population and stage. The levels of NO reached their maximum during stratification, which coincided with increased PTMs, and sharply declined upon germination. This dynamic redox transition is hypothesised to regulate the activity of key proteins involved in metabolic reactivation during seed germination. While the precise identities of modified proteins remain to be elucidated, our findings emphasize that NO-mediated PTMs are pivotal molecular signals that link environmental cues to physiological responses in seeds. It is hypothesised that these redox-based signaling mechanisms may be pivotal to local adaptation and contribute to the resilience of forest species in the face of climate change.

## additional info

This research was supported by the the National Science Centre, Poland (2019/33/B/NZ9/02660) and the Institute of Dendrology, PAS.

**Proteomics, metabolomics and phenomics**

**Oral presentation**

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# Integrating Mutant Approaches, Natural Variation, and Multi-Omics to Uncover the Role of Coumarins in Arabidopsis Stress Responses

Plants have developed various mechanisms to survive changing environmental conditions, such as drought, nutrient limitation, and pathogen attack. These adaptations have driven the evolution of diverse secondary metabolites and complex gene regulatory systems that enhance plant fitness and survival. Among these metabolites, coumarins play key roles in nutrient acquisition, environmental stress response, and shaping the root microbiome. Despite their importance, the full biosynthetic network underlying coumarin production in *Arabidopsis thaliana* is not yet fully understood. Here, we present an integrative approach to elucidate the coumarin biosynthesis pathway by combining Arabidopsis natural variation, mutant analyses, and multi-omics strategies. Through this approach, we have identified new components likely involved in the biosynthesis and exudation of secondary metabolites, including coumarins. We functionally characterized selected candidate genes encoding enzymes from the UDP-glucosyltransferase (UGT) and 2-oxoglutarate- and Fe(II)-dependent dioxygenase (2OGD) families using heterologous expression assays and phenotypic complementation studies. These results are supported by metabolomics, transcriptomics and gene expression analysis under Fe-deficient and osmotic stress conditions. Together, these data provide new insights into the regulation and diversification of coumarin metabolism in Arabidopsis and illustrate the power of combining classical genetics with multi-omics approaches to dissect complex secondary metabolic pathways in plants.

## *additional info*

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**Proteomics, metabolomics and phenomics**

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# Polyprenols, dolichols and their derivatives as biosynthetic intermediates, cofactors and modulators of biological membrane properties

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Polyisoprenoids (polyprenols and dolichols) - long-chain linear isoprenoid polymers - are found in every living cell. They are involved in many cellular processes and have various biological functions. They are cofactors in the processes of protein glycosylation, glucophosphatidylinositol anchor synthesis, and synthesis of bacterial cell wall polymers. Polyisoprenoids also act as modulators of the physical and chemical properties of cell membranes. In plants, deficiency of some polyisoprenoids is lethal, while the absence of others reduces the tolerance of plants to elevated temperatures. Deficiency of dolichols in humans leads to rare inherited diseases called CDG I (Congenital Disorders of Glycosylation type I). The Collection of Polyprenols, IBB PAS is a unique resource of natural isoprenoid (terpene) metabolites and their semisynthetic derivatives (e.g., prenyl aldehydes, phosphates, prenylammonium salts). In addition to polyprenols and dolichols, the Collection offers also oligoterpenes. These compounds can be used as standards in qualitative and quantitative determinations and as substrates in biochemical, biophysical and structural studies. The Collection of Polyprenols also offers support and consultation on analytical and preparative methods used in research focused on isoprenoids.

**Proteomics, metabolomics and phenomics**

**Oral presentation**

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# Adapting Crops for a Changing Climate

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Modern breeding focuses on speeding up heading to ensure grain yields amidst climate change. Heading date affects seed quality and is a key agronomic target. Our genetic work, in collaboration with international partners, accelerates breeding in barley and rice. We focus on floral-timing genes that determine when plants head, and traits like leaf/root architecture and tillering. Circadian-clock genes are key for transitioning crops from winter to spring, critical for adapting crops to warming climates. Collaborating with Vietnam (rice) and Germany (barley), we generate genomic circadian data, aiming to fine-tune cereal genetics for specific agroclimatic zones. The molecular genetics of flowering time in cereals, still emerging, has immediate relevance for pre-breeding and societal benefits.

**Application of Plant Biotechnology  
for Sustainable Agriculture**

**Keynote lecture**

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# What we have learned from carrot genome editing: from basic research to challenges for future applications

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Carrot (*Daucus carota*) has been widely used as a model species in numerous *in vitro* studies. We successfully applied CRISPR/Cas9 and Cas12a systems for carrot genome editing and mutant development. Several genes were targeted and mutated, resulting in robust protocols. These include stable transformation of hypocotyl explants and callus using Cas DNA vectors, as well as an alternative transient approach based on direct delivery of gRNA:Cas ribonucleoprotein complexes to protoplasts. The latter enables the generation of mutants free from foreign DNA, offering an advantage over transgenic lines with integrated Cas genes. Carrot genome editing involves biological and technical challenges relevant to applied research. *In vitro* systems expose issues such as genotype-dependent responses, low regeneration efficiency in some callus lines, somaclonal variation, chimerism, and delayed Cas activity. The biennial life cycle and complex seed production further hinder the development of stable mutants. Our results highlight the need for careful selection of genotypes and explants, optimisation of regeneration conditions, and long-term monitoring of mutation dynamics. Addressing these factors is essential to move beyond proof-of-concept studies and achieve practical applications of genome editing.

## *additional info*

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## **Application of Plant Biotechnology for Sustainable Agriculture**

### **Keynote lecture**

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# Targeting lipid degradation to enhance TAG accumulation in Arabidopsis Leaves

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In plants, triacylglycerols (TAGs) are the primary form of energy storage, typically accumulating in seeds within cytosolic lipid droplets (LDs). Interestingly, we observed also a progressive TAG accumulation during leaf senescence in both the dicot *Arabidopsis thaliana* and the monocot *Brachypodium distachyon*. Enhancing TAG synthesis in vegetative tissues is a promising strategy to increase the energy density of crop biomass. One approach involves a combined strategy: increasing fatty acid production (push), enhancing TAG assembly (pull), and reducing TAG degradation (protect). Our research focuses on the identification and functional characterization of key leaf lipases whose downregulation may promote TAG accumulation in leaves. Based on Arabidopsis transcriptomic data, we selected several candidate lipases and analyzed their impact on LD mobilization. To this end, we conducted a comprehensive analysis of lipid content and composition in selected lipase mutants during seed germination and leaf senescence. LD degradation was assessed by measuring the abundance of OLEOSIN1, the major structural LD protein. Additionally, LD localization and dynamics were examined using confocal laser scanning microscopy. Through this screening, we identified lipases whose loss of function delays LD degradation. These findings highlight specific lipases as promising targets for engineering enhanced TAG accumulation in vegetative tissues.

## *additional info*

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**Application of Plant Biotechnology for Sustainable Agriculture**

**Oral presentation**

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# Effect of Temperature Stress on Phenotype of *Arabidopsis thaliana* PDAT1 Mutant Lines

Phospholipid:diacylglycerol acyltransferase1 (PDAT1) is an enzyme that catalyzes the transfer of an acyl group from phospholipids to diacylglycerol, leading to the formation of triacylglycerol. While its primary role is storage lipid biosynthesis, PDAT also contributes to membrane lipid remodeling. This process alters fatty acid composition, which is crucial for maintaining plant homeostasis, particularly during stress conditions, by adjusting membrane structure. In our study we examined the effect caused by knock-out (KO) and overexpression (OE) of the PDAT1 gene in the model plant *Arabidopsis thaliana* under standard and stress temperature conditions – heat (35°C) and cold (6°C). Proteomic analysis of these lines cultivated under standard conditions revealed that OE lines were characterized by the upregulated level of protein involved in abiotic stress response, whereas KO lines showed the opposite effect. Our further study confirmed that PDAT1 overexpression boosts vitality in cold-exposed plants, stimulates their longevity and increases oil production at low temperatures, compared to wild-type and KO lines. The response to heat stress depended on its duration, generally causing opposite effect and impaired development of the overexpressing lines. These findings highlight the role of PDAT1 enzyme in plant adaptation to temperature stress and its contribution to seeds yield.

## additional info

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## Application of Plant Biotechnology for Sustainable Agriculture

### Oral presentation

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# Studies on the increased tolerance of apple polyploids to biotic stresses

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Polyploidization is a common phenomenon in plants with significant evolutionary importance and widespread application in plant breeding. Plant polyploids often exhibit superior traits compared to their diploid counterparts, such as larger leaves, flowers, and fruits, more vigorous growth, and increased adaptability to both biotic and abiotic stress factors. At the National Institute of Horticultural Research, thanks to an efficient *in vitro* polyploidization method, the numerous autotetraploids of six apple cultivars have been obtained. Preliminary assessments revealed that these tetraploid clones showed enhanced tolerance to biotic and abiotic stress factors compared to their diploid counterparts. The aim of the present study was to evaluate the susceptibility of selected tetraploid genotypes of the apple cultivars 'Redchief', 'Free Redstar', and 'Pinova' to infection by *Venturia inaequalis* and *Erwinia amylovora*, the casual agents of apple scab and fire blight, respectively. Results indicated that some tetraploid clones exhibited increased resistance to the diseases. To explore the mechanisms underlying this enhanced resistance, the expression levels of selected stress-related genes were analyzed in pathogen-inoculated plants. These included genes encoding antioxidant enzymes, pathogenesis-related (PR) proteins, and enzymes involved in the phenolic biosynthesis pathway. Additionally, biochemical markers of stress response in plants were examined.

## *additional info*

The study has been funded by the Polish Ministry of Agriculture and Rural Development as a grant Biological Progress in Crop Production, Task No. 49.

## **Application of Plant Biotechnology for Sustainable Agriculture**

### **Oral presentation**

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# BSA-seq approach identifies a genomic region associated with male sterility in sweet pepper

Cultivated pepper (*Capsicum annuum* L.) is one of the crop species in which male sterility systems have been successfully employed for hybrid seed production, and one of the systems is based on the nuclear ms8 gene, which has been mapped on chromosome P4. However, this gene has not yet been identified at the molecular level. This study aimed to identify the genomic region carrying the ms8 locus using bulked segregant analysis coupled with whole-genome resequencing (BSA-seq). To facilitate this analysis, an F2:3 segregating population was developed from a cross between the male-sterile ms8 line and the fertile line H3. Genomic DNA was extracted from the parental lines and F2 individuals, and two pooled DNA samples, consisting of fertile and male-sterile homozygotes, were prepared. These samples, along with the parental line DNA, were sequenced using the Illumina platform. Sequencing reads were aligned to two reference genomes, Zhangshugang and Zuhla-1\_v3.0, and single-nucleotide polymorphisms as well as insertions/deletions were identified. A bioinformatics analysis revealed the genomic region associated with the ms8 locus. This study provides novel insight into the genomic basis of male sterility in sweet pepper.

## additional info

Keywords: bulked segregant analysis, *Capsicum* genomics, genetic mapping, whole-genome resequencing

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## Application of Plant Biotechnology for Sustainable Agriculture

### Oral presentation

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# Light harvesting regulation and photodamage interplay in *Chlamydomonas reinhardtii*

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Photosynthesis is a critical process to all life on Earth, as it allows conversion of light energy and CO<sub>2</sub> into biologically-available compounds. Its integration in the metabolism of the cell and the variable nature of sunlight requires photosynthesis to be tightly regulated on various timescales. The failure to do so will result in transient or prolonged efficiency losses. In our group, we investigate the regulatory processes in photosynthesis, in particular concerning excitation energy transfer and electron flow. One of such mechanisms, of a particularly large extent in the green microalga *Chlamydomonas reinhardtii*, is state transitions. This process of antenna exchange between Photosystem I and II (PSII), upon various metabolic and light stimuli allows to balance light harvesting in the cells. I will present a combination of functional and ultrastructural data from a collaborative effort aiming at a description of the membrane-scale changes upon state transitions. Under extensive high light conditions this and other regulatory processes fail, leading to photodamage. Particularly concerning PSII, photoinhibition is a conserved process that is exacerbated by environmental stresses and limits photosynthesis; however, its molecular origins are still poorly understood. I will present methodologies developed in our group which aim at quantifying photoinhibition across photosynthetic phyla.

**Biology of chloroplasts and plant mitochondria**

**Keynote lecture**

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# The secrets of ribosomes and post-transcriptional events in plant mitochondria

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The ribosome is not only a protein-making machine, but also a regulatory element that can execute translational control. We discovered that deficiency of the ribosomal S10 protein in Arabidopsis mitochondria affects the biogenesis of mitoribosomes, leading to their distinct conformation and, in consequence, a subset of preferentially translatable mRNAs is changed. Unexpectedly, our data also suggest that several RNA maturation processes were significantly perturbed in mitochondria with altered ribosomes. We observed that a shortage of S10 protein exhibited a general decrease in efficiency of intron splicing, a defect in end-truncation and intercistronic processing of mRNAs, as well as rRNA processing. Our new results suggested that most of the observed changes in RNA metabolism in S10-deficient plants are associated with the changed activity of polynucleotide phosphorylase (PNPase), a crucial multifunctional enzyme in RNA homeostasis and decay in plant mitochondria. In this talk I will summarize our studies in which we have discovered that dysregulation of mitoribosome biogenesis affects the efficiency of mtPNPase in shaping the mature transcriptome. Our findings indicate a previously unappreciated interaction between the RNA translation and processing machines in plant mitochondria.

## *additional info*

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**Biology of chloroplasts and plant mitochondria**

**Keynote lecture**

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# Ultrastructural analysis of prolamellar body formation in oat leaves

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The prolamellar body (PLB) is a highly curved, complex, periodic membrane network that forms within the etioplasts of plants developing in darkness. It resembles an imbalanced diamond-type surface and serves as a precursor to the photosynthetically active thylakoids in chloroplasts. The PLB also acts as a storage compartment for lipids, proteins, carotenoids, and protochlorophyllide (Pchl<sub>ide</sub>). Although the light-induced transformation of PLBs into thylakoids has been well described, the mechanisms underlying PLB self-assembly remain elusive. To elucidate the ultrastructural stages of PLB formation in oat (*Avena sativa*), a representative monocot species, we conducted a transmission electron microscopy (TEM) study of membrane organization in proplastids and etioplasts from leaves of various lengths. Samples were collected along the entire leaf to capture the developmental gradient of plastid maturation characteristic of monocot leaves. Our observations revealed several precursor structures associated with PLB formation, including a sponge-like PLB structure (non-crystalline membrane network resembling mature PLBs) and parallel lamellae protruding from mature PLBs. The lamellae exhibited individual unit cells at their tips, which may serve as sites for further expansion of the crystalline PLB network. This study provides new insights into cubic membrane self-organization and highlights the diversity of PLB biogenesis pathways in monocots.

## *additional info*

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TEM was performed in the Laboratory of Electron Microscopy, Nencki Institute of Experimental Biology, using JEM1400 electron microscope (Jeol).

**Biology of chloroplasts and plant mitochondria**

**Oral presentation**

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# A large-scale study of chloroplast movements in wild plant species

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Chloroplast movements serve to optimize the photosynthetic efficiency of plants. In low light, chloroplasts relocate towards cell walls lying perpendicular to the direction of incident light to optimize light absorption. In high light, chloroplasts gather at cell walls parallel to the direction of incident light as a photoprotective mechanism. A distinct chloroplast position is observed in darkness. In angiosperm plants, chloroplast movements are mainly controlled by blue light photoreceptors, phototropins. In this work, a comprehensive analysis of chloroplast positioning was performed, with around six hundred wild-growing species of herbaceous plants investigated. Plants were collected in southern Poland from 2022-2025. They were growing in different light conditions, representing vegetation types from sun-exposed xeric grasslands, through mesophilic meadows, wetlands, ruderal communities, to the forest floor. Our results indicate that all major angiosperm families of Central Europe contain species that exhibit distinct chloroplast dark positioning. Chloroplast movements are more pronounced in plants collected in shade than in full sunlight, with a strong correlation between the magnitude of both responses. The amplitude of chloroplast responses also depends on the taxonomic affiliation of the species, with some families typically displaying substantial chloroplast movements regardless of the type of habitat they grow in.

## *additional info*

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**Biology of chloroplasts and plant mitochondria**

**Oral presentation**

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# Chloroplast intramembrane proteases: important factors for photosynthetic efficiency in *Arabidopsis thaliana*

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Efficient photosynthesis in plants relies not only on the structural integrity of chloroplasts but also on the precise regulation of their internal protein networks. A necessary and often overlooked key component of this regulation is the chloroplast-localized intramembrane proteases, particularly those belonging to the site-2 protease (S2P) family. In *Arabidopsis thaliana*, the proteases EGY1, EGY2, S2P2, and the pseudoprotease Egy3 each play distinct roles in maintaining chloroplast function and stability of the photosystem. EGY1 is essential for proper chloroplast development, fatty acid metabolism, and early gravitropic response. EGY2 influences chloroplast gene expression by modulating transcription-associated proteins. S2P2 is vital for maintaining appropriate levels of key proteins in photosystems I and II, as well as components of the light-harvesting complex. Although EGY3 lacks catalytic activity, it plays a regulatory role in stress adaptation by affecting proteome composition and redox homeostasis. Together, these proteases function as molecular architects of chloroplast organization and photosynthetic efficiency, both in control conditions and under abiotic stress. Understanding their roles provides valuable insights into plant stress resilience and the molecular mechanisms underlying chloroplast performance.

**Biology of chloroplasts and plant mitochondria**

**Oral presentation**

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# Photoprotective role of PsbS and zeaxanthin in seedlings of *Arabidopsis thaliana npq1* and *npq4* mutants

The photosynthetic complexes must efficiently adapt to changing light conditions and be protected from excess light. Non-photochemical quenching (NPQ) protects plants by dissipating excess excitation energy as heat. The fastest NPQ response is triggered by lumen acidification in light and involves PsbS protein and zeaxanthin formation. This has already been demonstrated for adult *Arabidopsis* plants (1-2 months) [1]. We focused on seedlings – a stage at which the establishment of photosynthesis is particularly sensitive to light stress. We examined the photosynthetic performance in *Arabidopsis thaliana* mutants: *npq1* (lacking functional VDE) and *npq4* (deficient in functional PsbS protein). Analyses were performed using *in vivo* chlorophyll fluorescence imaging, including analyses of NPQ induction and relaxation kinetics. Additionally, 77 K low-temperature fluorescence spectroscopy was used to examine PSI/PSII antenna organization. Pigment composition (xanthophyll cycle activity and chlorophylls content), was determined by high-performance liquid chromatography (HPLC). This approach revealed how the absence of zeaxanthin or PsbS affects NPQ dynamics, pigment accumulation, and photosynthetic performance during early plant development. Importantly, comparisons were made at comparable developmental stages rather than by age, as mutants may differ in growth rate. This allowed for a more accurate assessment of NPQ function in young plants.

## additional info

[1] Ware MA, Belgio E, Ruban AV (2014) Comparison of the protective effectiveness of NPQ in *Arabidopsis* plants deficient in PsbS protein and zeaxanthin. *Journal of Experimental Botany*, 66, 1259–1270.

**Biology of chloroplasts and plant mitochondria**

**Oral presentation**

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# The mitochondrial NDA2 dehydrogenase as a key regulator of Arabidopsis responses to abiotic stresses

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Plant mitochondria, through the engagement of alternative pathways in the mitochondrial electron transport chain (mtETC), including alternative oxidase (AOX) and type II NAD(P)H dehydrogenases (NDH-2), are proposed to be key regulators of redox balance, energy homeostasis, and stress responses. The aim of the research was to examine how NDA2 overexpression (a matrix-facing NDH-2) affects *Arabidopsis thaliana* metabolism under control and abiotic stress conditions, including nutritional, drought and low-temperature stresses. Under optimal growth conditions, oeNDA2 plants showed a decline in the leaf tissue redox state, but no alteration in energy homeostasis. However, detailed transcriptomic (microarray and RT-qPCR), proteomic (BN- and 2D-analyses), and/or metabolomic (spectro- and luminometric) analyses indicate that the mutants respond differently to stress factors compared to wild-type plants, exhibiting increased (in the case of drought) or decreased (in the case of ammonium nutrition) resistance. Interestingly, differences in stress responses between the genotypes appear, despite the fact that NDA2 overexpression was balanced by a reduction in the expression of its homolog, NDA1. Overall, our findings suggest that NDA2 plays a significant role in Arabidopsis adaptation to stress conditions.

## *additional info*

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**Biology of chloroplasts and plant mitochondria**

**Oral presentation**

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# A lateral inhibition pathway regulates Arabidopsis body axis formation

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Formation of the main body axis is one of the earliest developmental decisions during plant and animal embryogenesis. In the flowering plant *Arabidopsis*, apical-basal axis formation is evident at the asymmetric zygote division that segregates two daughter cells with different transcriptional programs and developmental perspectives. The biparentally activated WRKY2/WOX8 transcription factors regulate zygote polarity. After the zygote division, polar transport of the phytohormone auxin underlies axis formation. However, the connection between these principal pathways has remained enigmatic. Here, we discuss how the WRKY2/WOX8 pathway links the regulation of zygote polarity to stable *Arabidopsis* axis formation.

**Advances in plant structure and development**

**Keynote lecture**

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# The endomembrane system in plant developmental patterning

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Plant development is instructed by the hormone auxin. Polar cell-to-cell auxin transport has a major role in auxin distribution on tissue scales, resulting in the formation of auxin maxima and channels that act as blueprints for the patterning of organs and internal structures, e.g. vasculature. On the level of individual cells, auxin-mediated development is under regulation by the ARF small GTPase machinery, which is a conserved component of eukaryotic endomembrane systems. Beside housekeeping functions in vesicular trafficking, selected ARF machinery components in plants, represented by the ARF-GEF regulator GNOM, evolved pivotal roles in auxin-mediated development through a mechanism that includes, but is not limited to, the regulation of PIN auxin efflux carrier polarity and transport activity. Despite decades of study, the exact mechanism of GNOM function as a developmental patterning regulator is not fully understood. I will present recent steps towards the elucidation of GNOM molecular mechanism, capitalizing on GNOM's nature as an ARF-GEF endomembrane system component and on its functional divergence from its close homologue in *Arabidopsis thaliana*, GNOM-LIKE1. I will also discuss future plans of my group aimed at advancing the understanding of GNOM mechanism and of the evolution of this unique ARF-GEF within the plant lineage.

## *additional info*

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**Advances in plant structure and development**

**Keynote lecture**

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# Uncovering genes under MONOPTEROS regulation during the embryogenic transition in *Arabidopsis thaliana*

The process of somatic embryogenesis (SE), mainly induced by auxin, leads to the formation of bipolar structures called somatic embryos. Studies on the process of *Arabidopsis thaliana* SE contribute to identifying key components that turn on the embryogenic developmental pathway, i.e., transcription factors, miRNAs, or epigenetic markers. One of the genes playing a key role in the SE is the MONOPTEROS (MP) gene. MP undergoes the strongest expression during the *Arabidopsis thaliana* SE process. Additionally, the MP isoform, called MP11ir, was identified as being involved in SE. The MP11ir transcript, an alternatively spliced variant of MP, produces a truncated protein missing the PB domain, crucial for repression of MP by Aux/IAAs. Both MP and MP11ir are essential for embryo regeneration in the mp mutant. However, overexpressing truncated MP protein ( $\Delta$ ARF5) lacking the PB1 domain inhibits SE, leading to callus formation instead of somatic embryos. The precise mechanism of MP and MP11ir action in SE is unknown. Transcriptome analysis was done in different transgenic lines to learn about the MP and MP11ir mechanism of action. The study identified a set of genes potentially under MP and/or MP11ir control.

## additional info

This research was funded in part by the National Science Centre, Poland, under the OPUS call in the Weave programme (2023/51/I/NZ1/00704).

**DNA and RNA metabolism, epigenetics and genomics**

## Oral presentation

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# Annexin 5 is a novel regulator of ribosomal RNA reserves accumulation during pollen development in *Arabidopsis thaliana*

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Mature pollen grains, like dry seeds, store large reserves of RNA species that enable rapid activation and germination under favorable conditions. In *Arabidopsis thaliana*, the gene encoding annexin 5 (ANN5) is activated in bicellular pollen; however, its specific role at this stage remains unclear. We have demonstrated that ANN5 is primarily associated with the nucleolus, where it influences ribosomal RNA (rRNA) metabolism at multiple levels. Depletion of ANN5 contributes to a significant decrease in the level of stored rRNA in mature pollen grains. Using a three-component BiFC assay, we found that ANN5 can influence the organization of the HD2B/RPS6 complex, which may affect the expression of rRNA gene variants and/or the maturation of rRNA. Moreover, the opposing effect of ANN5 on the nucleolar distribution of RPS6 paralogs indicates that RPS6A may be preferentially directed to the multimeric HD2B protein complex, while RPS6B may be directed to the cytoplasm. This may lead to RPS6 paralog sorting, which could affect the composition of ribosomes stored in mature pollen. We propose that the main physiological role of ANN5 is to promote the accumulation of rRNA reserves in developing pollen grains, whose activation ultimately determines the pollen grain's competitiveness on the stigma.

## additional info

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**Advances in plant structure and development**

**Oral presentation**

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# In search of relationships between hypocotyl growth, mechanics and hemicelluloses of primary cell walls

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Hemicelluloses are one of three major polysaccharides of plant cell walls. They contribute to the mechanics and integrity of secondary cell walls. However, their role in the primary cell walls, especially the wall expansion and mechanical properties, has not yet been fully understood. Hemicelluloses comprise a diverse group of polysaccharides that are known for their ability to interact with cellulose microfibrils. This study aims to verify the hypothesis that hemicelluloses affect the mechanical properties and growth of primary cell walls of elongating conifer hypocotyls. We use enzymatic treatment of hypocotyls of spruce *Picea abies* and pine *Pinus sylvestris*, and perform mechanical tests using extensometer to assess their tensile stiffness, and various cutting experiments to reveal a pattern of tissue stresses. Immunolabeling with specific antibodies is used to localise the different hemicelluloses in hypocotyl cell walls. Such an integrated approach provides insights into the hemicelluloses' contribution to the mechanical properties of the primary cell walls in conifers.

## additional info

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**Advances in plant structure and development**

**Oral presentation**

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# Charge of the Plant Extracellular Matrix and Its Role in Calcium Homeostasis

The plant extracellular matrix (ECM), also referred to as the cell wall, is a complex, dynamic structure composed of polysaccharides and proteins, that serves as the initial point of contact with the environment and regulates the entry of ions and other substances into cells. Pectin comprises up to 30% of the cell wall. Once in the cell wall, pectin is conditionally processed by enzymes of the PECTIN METHYL ESTERASE (PME) family, which remove methyl groups and leave behind negatively charged carboxyl groups—thus contributing to the electronegativity of the ECM. It enables cross-linking with divalent cations such as calcium ( $\text{Ca}^{2+}$ ), enhancing cell wall strength and rigidity. Conversely, enzymes from the PME-INHIBITOR (PMEi) family suppress PME activity, thereby maintaining a low proportion of un-esterified pectin and reducing cell wall electronegativity. Our data indicate that calcium availability affects cell wall charge and the expression of genes encoding cell wall modifying enzymes. Consecutively, cell wall charge influences both calcium acquisition and apoplastic free  $\text{Ca}^{2+}$  levels, as well as growth fitness under calcium-deprived conditions. Taken together, our findings support the hypothesis that plants modulate ECM charge in a PME-dependent manner as an adaptive strategy to cope with varying calcium availability.

## *additional info*

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**Advances in plant structure and development**

**Oral presentation**

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# Molecular Insights into VND-Mediated Cell Wall Formation in Conifers

Conifer wood consists of tracheid cells surrounded by thickened cell walls (CWs), composed of cellulose, hemicelluloses, and lignin. Interestingly, seasonal wood formation results in earlywood and latewood with distinct CW architectures and biochemical profiles (1). To explore the genetic basis of these biochemical differences, we identified *Picea abies* VND (VASCULAR-RELATED NAC-DOMAIN) transcription factor homologs (PaVNDs). We confirmed their role in CW deposition and revealed light-dependent PaVNDs expression during wood transition. PaVND functions were assessed by transient transformation of *N. benthamiana* and resulting CW analysis. Biochemical and gene expression analyses showed that each PaVND differentially regulates CW formation. Raman spectroscopy revealed distinct CW assembly dynamics associated with specific PaVND activity. Further experiments suggested that, PaVND activity may be influenced by redox homeostasis and protein interactions. Expanding CW studies beyond dicot models, we initiated a CW induction system in gymnosperm protoplasts to provide a novel model for conifer xylogenesis studies. To support comparative studies across species, we developed software enabling multi-species BLAST analyses, result validation, and integrated filtering, facilitating preparation of datasets for phylogenetic and functional analyses. Our findings improve understanding of CW transcriptional control, paving the way for wood modification strategies and providing new insights into PaVND function in CW composition.

## additional info

(1)Liszka et al. (2023) Structural differences of cell walls in earlywood and latewood of *Pinus sylvestris* and their contribution to biomass recalcitrance. *Front. Plant Sci.* 14:1283093

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# Light signal transduction from shoot to root at low temperatures in cereals

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In order to better understand the role of light in cold acclimation processes, 3 approaches were used. In the 1<sup>st</sup> experiment, rice plants were exposed to low temperatures with different light intensities. In the case of some plants, only the leaves received the cold, while the roots remained at the control temperature. Using the RNAseq technique, the comparison of the leaf-stressed plants with either the whole cold-treated or the control plants revealed that nitrogen metabolism and nitric oxide-related signalling, and the phenylpropanoid-related processes were specifically affected. Light conditions may also affect certain stress responses in the roots at gene expression and metabolite levels. In the 2<sup>nd</sup> experiment, wheat plants were grown under white or blue light conditions, then plants were exposed to cold. Chlorophyll-*a* fluorescence, targeted and untargeted HPLC analyses and gene expression analyses demonstrated that blue light may induce specific cold acclimation processes in wheat leaves, and the effects of light signal can also be detected in roots. In the 3<sup>rd</sup> experiment, young maize plants were cold acclimated at different light intensities. After that, not only its effect on cold stress responses, but also the responses to another stressor, high salinity were analysed at metabolite and gene expression levels.

## *additional info*

This work was supported by the National Research, Development, and Innovation Office (grant TKP2021- NKTA-06).

## Plants and abiotic stresses

### Keynote lecture

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# Abiotic Stress Signaling– Follow the SnRK2 Pathways

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Salinity and drought are the most common abiotic stresses that plants face. To detect and respond effectively to these challenges, plants have evolved various signaling pathways, in which protein phosphorylation and the formation of biomolecular condensates play a crucial role. SNF1-related protein kinases 2 (SnRK2) are key regulators of plant responses to abiotic stresses. All are activated by osmotic stress but differ in their response to abscisic acid (ABA). ABA-activated SRK2 kinases have been most extensively studied, as they are part of the core ABA signaling pathway. In contrast, far fewer studies have been conducted on the ABA-insensitive SnRK2 kinases. Our group's research is focused on these kinases. Recently, we identified several targets of the ABA-insensitive SnRK2 kinases, among them glycine-rich RNA-binding protein 8 (GRP8). GRP8 negatively affects root growth and seed germination under salt stress conditions. Under salinity stress, GRP8 is phosphorylated within its RNA Recognition Motif (RRM) and is recruited to stress granules (SGs). *In vitro* studies demonstrate that this phosphorylation significantly alters GRP8 structural dynamics, facilitating GRP8 dimerization and subsequently liquid-liquid phase separation, thereby promoting its assembly into SGs. The results from our group and others demonstrate that SnRK2s regulate abiotic stress responses at various levels.

**Plants and abiotic stresses**

**Keynote lecture**

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# The cost of survival: Barley HvD53A mutation boosts drought tolerance at the expense of photosynthesis

Strigolactones (SLs) are plant hormones that shape architecture and help plants adapt to environmental stress. In this study, we examined the effects of a mutation in a component of the barley SL signaling pathway, the SL repressor HvDWARF53A, on plant growth and drought tolerance. We compared the results with those of a previously described barley mutant, highly tillered and drought-sensitive, carrying a mutation in the SL receptor gene HvDWARF14. The two mutants, hvd14.d and hvd53a.f, displayed contrasting phenotypes, including differences in plant height, tillering, and drought sensitivity. Ultrastructural analysis revealed smaller chloroplasts and fewer grana stacks in hvd53a.f under control conditions, possibly explaining its lower photosynthetic efficiency. Transcriptomic analysis linked upregulated genes with antioxidation and stress responses, suggesting improved drought resilience. Further analysis showed a link between SL signaling and circadian components, with CIRCADIAN CLOCK ASSOCIATED1 emerging as a potential SL-responsive regulator of tillering. Under drought, hvd53a.f exhibited enhanced tolerance and stable, albeit reduced, photosynthetic performance. We also identified the SL-related TF JUNGBRUNNEN1 as a potential regulator of genes involved in water deficit response and antioxidation. Overall, the hvd53a.f mutation enhances drought tolerance while maintaining low, stable photosynthesis, highlighting HvD53A as a central node connecting SL signaling to stress resilience.

## *additional info*

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## Plants and abiotic stresses

### Oral presentation

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# DNA methylation signatures of drought stress priming in potato cultivars

Drought stress threatens the productivity of potatoes worldwide. We investigated whether a drought event “priming” could induce stress memory, affecting tuber yield and DNA methylation in successive generations. In our experiment, Katahdin and five derived cultivars underwent one drought episode and were then grown under control conditions for two subsequent tuber generations. We measured tuber yield and profiled genome-wide DNA methylation. Primed plants had significantly lower yields in the first generation, with only partial recovery in the second generation. Methylation profiling revealed widespread, cultivar-specific changes in the first progeny; each cultivar exhibited unique differentially methylated regions (DMRs) after priming. Importantly, these epigenetic marks were transmitted to the first progeny but were largely reset by the second generation. Our findings highlight transgenerational drought memory via DNA methylation in potato, with cultivar-specific responses. Understanding this epigenetic memory could guide the breeding of drought-resilient potato varieties.

## *additional info*

This work was supported by National Science Centre in Poland, under Grant No UMO-2020/37/B/NZ9/00028.

## Plants and abiotic stresses

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# TATOOINE: A novel chloroplast-localized protein involved in plant drought response

Our research focuses on TATOOINE (AT4G25290), a chloroplast-localized protein from *Arabidopsis thaliana*, whose overexpression enhances drought tolerance while increasing biomass. During drought plants overexpressing TATOOINE (OE) flower earlier than wild type (WT) and tatooine knock-out mutants. This accelerated seed production likely ensures species survival. Notably, only OE plants recover after severe drought. To explore the molecular role of TATOOINE we analyzed the expression of ABA-dependent signaling genes, as ABA is a key hormone involved in plant stress response. Preliminary results showed that plastoglobules of OE plants have different MGDG:DGDG ratio than WT and tatooine. Plastoglobules are lipoprotein structures present in chloroplasts, significant to thylakoid function. MGDG and DGDG are the main lipids in thylakoid membranes. Their proper ratio is important for different cellular processes. Microscopic observations revealed that tatooine mutant is not able to properly metabolize lipids in the plastoglobules. We hypothesize that TATOOINE can improve drought tolerance through regulating lipid metabolism. We showed that TATOOINE belongs to a novel plant-specific protein group with photolyase and hydrolase domains. These proteins remain uncharacterized in the literature. Our findings provide new insights into plant drought adaptation strategies, laying the foundation for future studies and potential agricultural applications.

## *additional info*

The research was supported by the Polish National Science Centre, grant no. UMO-2016/22/E/NZ3/00326.

## Plants and abiotic stresses

### Oral presentation

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# Novel O-demethylation activity leads to esculetin biosynthesis and impacts stress response

Coumarins like scopoletin and esculetin are secondary metabolites playing roles in plant response to abiotic and biotic stresses, including iron deficiency and pathogens. Many stages of the coumarin biosynthetic pathway remain unclear. We identified a previously undescribed *Arabidopsis* gene group encoding 2-oxoglutarate and iron-dependent dioxygenases (2OGD) sharing sequence similarity with 2OGDs catalyzing O-demethylation in opium poppy morphine biosynthesis pathway. We overexpressed the proteins encoded by the candidate *Arabidopsis* 2OGD genes and conducted enzymatic characterization. We confirmed activity towards various compounds involved in the coumarin biosynthesis pathway. Two proteins, which we named S6OD1 and S6OD2, catalyze *in vitro* O-demethylation of scopoletin to form esculetin. Metabolomic results show that at high pH in the *s6od1* plants roots the accumulation of esculetin is lower and scopoletin higher than in Col-0 WT. We observed lower concentrations of esculetin and esculin for the mutant grown in soil, hydroponics and *in vitro*. The *s6od1* mutants also show impaired response to cold and higher susceptibility to *Dickeya* spp. infection. We show for the first time O-demethylation activity in *Arabidopsis* and elucidate the biosynthesis of esculetin. O-demethylation being present in a widespread metabolic pathway suggests it is a more common reaction type among plants than was previously acknowledged.

## additional info

Funding: NCN grant UMO-2022/47/B/NZ2/01835 (AI) and NAWA grant BPN/BFR/2022/1/00039.

## Plants and abiotic stresses

### Oral presentation

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# Small RNAs and Argonautes in Plant-Virus Interactions: Mechanistic Insights and Biotechnological Applications for Precision Crop Protection

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Small RNAs (sRNAs) and their effector proteins, the Argonautes (AGOs), form the core of plant RNA-silencing pathways that detect and neutralize invading viruses. Antiviral AGOs load virus-derived sRNAs to repress complementary viral RNAs or DNA, or with endogenous sRNAs to regulate host gene expression and promote antiviral defense. In turn, viruses can be manipulated to be used as viral vectors for expressing highly specific artificial sRNAs that recruit endogenous AGOs to knock down selected genes. Remarkably, viral vectors can be applied in a transgene-free manner to produce *in vivo* a well-defined set of highly specific synthetic trans-acting small interfering RNAs (syn-tasiRNAs) for providing strong immunity against pathogenic viruses. Together, these strategies show how plant-virus interactions mediated by sRNAs and AGOs can be reprogrammed for precision crop protection.

**Plant biotic interactions**

**Keynote lecture**

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# Cytokinins in and out - how ABC driven transport shapes Medicago root morphology upon nitrogen deficiency and interactions with Rhizobia

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Legumes symbiotically interact with rhizobium bacteria, utilizing cytokinins (CKs) as key factors in nodule inception and efficient growth on nitrogen-deprived soils. Despite numerous studies indicating a significant role of CK distribution for legume root morphology and symbiotic interactions, our understanding of the underlying mechanism of CK transport remains limited. We have identified and characterized two *Medicago truncatula* full-size ATP-binding cassette (ABC) proteins of the G subfamily, namely ABCG40 and ABCG56, as CK transporters. Their expression is root-specific and is induced by nitrogen shortage and rhizobia. The MtABCG40 contributes to the horizontal translocation of CKs from xylem to the cells originating lateral organs. As such, it impacts root morphology, negatively affecting lateral roots and nodule formation upon nitrogen shortage. The MtABCG56, exports active CKs produced in the epidermis in response to microsymbionts to the cortex. Consequently, the CK signalling pathway is activated in the inner cortical layers, promoting cell divisions and the formation of root nodule primordia. Together those transporters represent an example of functional specialization among members of this multigenic family and allow us to propose model of CKs distribution in *M.truncatula* root.

**Plant biotic interactions**

**Keynote lecture**

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# Type three effector HopBF1 of *Pseudomonas syringae* induces systemic micronecroses in *Nicotiana benthamiana*

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The *Pseudomonas syringae* effector protein HopBF1 is an atypical kinase that inhibits the host chaperone HSP90. HopBF1 phosphorylates HSP90 in a conserved serine residue that interferes with the ATPase activity of the chaperone. While its local activity causes tissue collapse, HopBF1 also induces cell death in distant, systemic leaves where the effector mRNA or protein is not detected. This suggests a generation of a mobile signal from local to distant leaves. To investigate the underlying mechanism, we performed a transcriptomic analysis of both local systemic leaves. We observed significant upregulation of genes associated with the unfolded protein response (UPR), endoplasmic reticulum (ER) stress, and autophagy in local leaves. On the other hand, the systemic leaves showed a transcriptomic profile reminiscent of defense-related programmed cell death. Based on these findings, we propose a model where HSP90 inhibition by HopBF1 leads to a critical accumulation of misfolded proteins. This overwhelms the UPR, triggering pro-cell death autophagy. The resulting cytotoxic byproducts may then move through the vasculature, causing the observed systemic necroses. To our knowledge, this is the first report of a bacterial type three secreted effector inducing a systemic response without its physical presence in the affected tissue.

## additional info

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## Plant biotic interactions

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# Nod factor perception and signal transduction during endosymbiotic interactions of *Medicago truncatula*

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The legume *Medicago truncatula* maintains endosymbiotic interactions under phosphate and nitrate limiting conditions with respectively mycorrhizal fungi and rhizobia. Both microsymbionts produce lipochitooligosaccharides (LCOs) which are perceived by plant receptors with extracellular LysM domains and an intracellular kinase domain (LysM-RLKs). Mycorrhizal fungi in addition secrete short chain chitooligosaccharides (COs) which also function as signal molecules. Three LysM-RLKs from *M. truncatula*, LYR3, LYK3 and NFP, were studied in detail in our group. LYR3 is a high affinity binding protein for LCOs but not for COs. LYR3 and NFP lack an active kinase domain and LYR3 is phosphorylated by the active kinase domain of LYK3. FRET experiments showed that LYR3 and LYK3 interact at the plasma membrane and this interaction is inhibited or disrupted by addition of LCOs. Co-expression of NFP and LYK3 in tobacco leaves provokes a cell death response that is attenuated in the presence of LYR3. Thus, LYR3 may play a role in regulating the functional interaction of NFP and LYK3. Low level expression of these 3 proteins hampers their visualization in roots of *M. truncatula*. Ongoing experiments to increase the detection sensitivity and thus study the distribution and regulation of these proteins during endosymbiotic interactions will be presented.

**Plant biotic interactions**

**Oral presentation**

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# Aphid-Mediated Virus Infection Alters Volatile Organic Compound Emissions in the Pepper plant

Insect vector-borne viruses can alter the phenotype of host plants to manipulate their response to biotic stress. Under such stress, plants emit volatile organic compounds (VOCs) as part of their defense strategy. However, viral infection can modify VOC profiles, enhancing host-vector interactions and promoting virus dissemination, often resulting in significant crop yield losses. This study investigates virus-induced VOCs in pepper (*Capsicum annuum*) infected with *Pepper Vein Yellow Virus-2* (PeVYV-2) and evaluates their potential as biomarkers for early detection and pest management. We hypothesize that virus-infected pepper plants emit a distinct volatile spectrum that influences the behavior of *Myzus persicae*, the virus vector. VOCs were extracted using headspace solid-phase microextraction (HS-SPME) and analyzed via gas chromatography-mass spectrometry (GC-MS). Comparative analysis revealed elevated levels of green leaf volatiles (GLVs) and fatty acid derivatives in both non-viruliferous and viruliferous aphid-infested plants. In contrast, terpenoids, phenylpropanoids, and benzenoids were significantly reduced in both infested treatments compared to healthy controls, suggesting suppression of defense-related signaling. These VOC changes likely enhance vector attraction and virus spread. This research offers new insights into pepper-aphid-virus interactions and highlights the value of VOC profiling in the development of sustainable, VOC-based crop protection strategies.

## additional info

We acknowledge Ben-Gurion University of the Negev, ISF, and MERC for supporting this project.

## Plant biotic interactions

## Oral presentation

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# C-terminal part of phytochelatin synthase supports its function in pathogen-triggered indole glucosinolate metabolism in Brassicaceae

Phytochelatin synthase (PCS) is primarily known for enabling plant tolerance to heavy metal ions via phytochelatin (PC) synthesis. Recent studies revealed additional function of PCS1 from *Arabidopsis thaliana*, contribution to the PEN2-dependent indole glucosinolate (IG) metabolic pathway, critical for defence against filamentous pathogens. This function is distinct and independent from PCS role in PC biosynthesis, though its molecular basis remains unclear. To uncover this, we explored whether PCS1 orthologs from Brassicaceae species that possess (*Cardamine hirsuta*) or lost (*Camelina sativa*, *Capsella rubella*) PEN2 pathway can substitute for AtPCS1 in pathogen triggered IG-metabolism. We expressed these orthologs and chimeric constructs in pcs1 mutant background under the native AtPCS1 promoter. IG metabolic activity was tested by measuring accumulation of PEN2/PCS1-dependent end products after elicitor treatment. Our analysis showed that ChPCS1, but neither CsPCS1 nor CrPCS1, complement efficiently pcs1 mutant restoring accumulation of these compounds. This indicated that PCS1 isoforms from IG-deficient species are not functional in IG metabolism due to mutations in amino acid residues important for this function. Moreover, investigation of chimeric AtPCS1/CrPCS1 revealed that C-terminal PCS1 part is indispensable for IG metabolism. Overall, these findings support identification of residues critical for the investigated novel function of PCS1.

## additional info

This research is supported by the National Science Centre, Poland as an OPUS 20 grant: 2020/39/B/NZ2/03426.

## Plant biotic interactions

## Oral presentation

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# Using GWAS and the *Arabidopsis–Dickeya* pathosystem to elucidate links between iron nutrition, coumarins, and plant defense

As shown previously by our research group, *Arabidopsis thaliana* is an excellent model to study the coumarin biosynthetic pathway and the natural variability of coumarin accumulation in different environments. To better understand the link between maintenance of iron (Fe) homeostasis, biosynthesis and accumulation of coumarins, and plant immunity, we exploit genetic variability among natural *Arabidopsis* populations and study their differential susceptibility to plant pathogens of the genus *Dickeya* spp. under various Fe availability. We cultivated 105 *Arabidopsis* accessions in hydroponic cultures under Fe-deficient conditions both with and without pathogen inoculation, and collected a phenotypic dataset focusing on traits such as root and shoot fresh weight, pigments quantity, disease severity index, and systemic response at different time points. In parallel, metabolic profiling is ongoing to quantify coumarins in roots and root exudates, as well as ionomics to determine Fe and other nutrients levels. To identify the genetic variation associated with these traits, Genome-Wide Association Studies (GWAS) are underway and the first results have been obtained. Our goal is to identify candidate genes associated with the observed phenotypic variability, revealing new components of the coumarin synthesis pathway involved in the ‘battle for iron’ between plants and pathogens.

*additional info*

Funding: NCN grant UMO-2022/47/B/NZ2/01835 (Anna Ihnatowicz).

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**Plant biotic interactions**

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# Plasmodesmata cell walls: the mechanical and structural properties that control communication

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Plasmodesmata provide a route for the transport of small and large molecules including signalling proteins and RNAs, metabolites and hormones. The cell walls surrounding plasmodesmata regulate their connectivity and are enriched in the  $\beta$ -1,3 glucan polysaccharide callose. We have studied the properties and function of callose in the regulation of the channel structure and identified enzymes that modify its accumulation. A combination of AFM- nanoindentation and texture analysis have been used to investigate the mechanical properties of cell walls around plasmodesmata and to correlate these parameters with callose accumulation and the expression of callose-regulatory proteins. This research revealed structural properties of these cell walls microdomains that can be exploited in modifying fruit development and in biomaterial development. I will share how we build on this knowledge to understand the potential benefits in engineering this mechanism.

## *additional info*

The author acknowledge the contributions of the whole Benitez-Alfonso team at Leeds and collaborators. Funding to support the lab work is provided by UK Research and Innovation council Future Leaders fellowship (MR/T04263X/1).

**Short- and long-distance communication in plants**

**Keynote lecture**

**Authors:**

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# Small but powerful - plant gasotransmitters in (mode of) action

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Signal transducers, gasotransmitters, are small molecules with pleiotropic biological functions in plant growth and development. Endogenously synthesized, they mediate several physiological functions using specific molecular targets and messengers. These compounds usually characterise the bimodality of the action. They play a regulatory role in low concentrations. Plants can use their toxic content as a biological “chemical bomb”, a defensive mechanism against stressors, mainly biotic. Hence, gasotransmitters’ current concentration is tightly regulated. These compounds include more or less known molecules, like nitric oxide (NO), hydrogen sulphide (H<sub>2</sub>S), carbon monoxide (CO), but also hydrogen cyanide (HCN), phytohormone ethylene (ET, C<sub>2</sub>H<sub>4</sub>), molecular hydrogen (H<sub>2</sub>), and methane (CH<sub>4</sub>). While there is growing interest in these molecules, their metabolism, signalling pathways, and cross-talk remain little known. The aim of this presentation is to focus on NO, H<sub>2</sub>S, HCN, and ET metabolism, the cellular network of connections in plants under physiological and pathophysiological conditions.

**Short- and long-distance communication in plants**

**Keynote lecture**

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# Structural Pathways and Functional Implications of Cell-to-Cell Communication in *Arabidopsis thaliana* Male and Female Germ Units

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The unique structural organization of the male germ unit (MGU), consisting of two sperm cells and the vegetative cell nucleus, and the female (FGU) germ unit, comprising the synergids, egg cell and central cell, is critical for successful sexual reproduction in *Arabidopsis thaliana*. We identified specialized cellular connections that facilitate communication within these reproductive units using light and electron microscopy. In the FGU, we observed potential symplasmic connections between egg and central cells, with an irregular and discontinuous extracellular matrix between the cells that brings their plasma membranes into direct contact at specific regions. Through multiple staining approaches and immunocytochemistry, we demonstrate that the extracellular matrix surrounding the FGU cells has a unique composition distinct from the typical plant cell wall. Within the MGU, we documented a physical association between one sperm cell and the vegetative nucleus, where the sperm cell's cytoplasmic projection becomes enveloped by the lobed structure of the vegetative nucleus. This association enables coordinated movement of sperm cells within the growing pollen tube but also facilitates direct communication between MGU cells. These physical connections between cells in MGU and FGU, provide structural routes for potential symplastic transport and cell-cell communication, underlying documented molecular exchange between these cells.

**Short- and long-distance communication in plants**

**Oral presentation**

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# Dolichol biosynthesis mutant *lew1* affects pollen development and plant fertility in *Arabidopsis*

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Cell-cell recognition and in particular pollen-ovule recognition rely to a large extent on proper glycosylation of extracellular proteins that interact during pollen-pistil recognition, pollen tube growth, and ovule fertilization. Dolichol phosphates are obligatory cofactors in the protein glycosylation process and the synthesis of the glycosylphosphatidylinositol anchor of extracellular proteins. Dolichol phosphates are synthesized in all eukaryotic cells in the endoplasmic reticulum by an iterative elongation of a short allylic precursor farnesyl diphosphate with the isopentenyl diphosphate molecule, followed by dephosphorylation, chemical reduction of the double bond closest to the hydroxyl group, and phosphorylation again. The first of the mentioned processes is catalyzed by an enzymatic complex consisting of an enzyme *cis*-prenyltransferase CPT, and its accessory protein LEW1. Here we describe the genetic and cellular effects of LEW1 deficiency on *Arabidopsis thaliana* pollen development and fertility.

*additional info*

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**Short- and long-distance  
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**Oral presentation**

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# Role of MOTHER OF FT AND TFL1 in Seed Development and Germination in *Medicago truncatula*

Seeds are highly specialised structures that enable survival of plant progeny in variable climates. The conditions encountered by mother plants determine a number of factors in seeds, one of which is dormancy depth. Deep dormancy prevents germination even if all the proper external conditions are met. The transition between dormancy and germination of mature seeds is regulated by the interplay of abscisic acid (ABA) and gibberellin (GA) pathways. One of the components of the ABA and GA signaling pathways recruited to control seed germination is the MOTHER OF FT AND TFL1 (MFT) protein. We have shown that MFT from *Medicago truncatula* is highly expressed in developing seeds and in the embryonic root of mature seeds. The expression of MtMFT in imbibed seeds is related to the ABA pathway and dependent on the current level of dormancy. Furthermore, freshly harvested mtmft seeds exhibited significantly higher germination rates compared to WT seeds. Our results also suggest MtMFT's role in so called "thermoinhibition" of seeds, showing its importance in various biological scenarios.

## additional info

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**Short- and long-distance communication in plants**

**Oral presentation**

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# When hormones meet signal molecules - understanding strigolactone and cNMP crosstalk in plants

Strigolactones (SLs) are carotenoid-derived plant hormones that regulate diverse developmental and environmental responses, including shoot branching, root architecture and adaptation to phosphate/nitrogen deficiency, drought and salinity. SL perception begins with binding of the ligand to the D14 receptor, followed by activation of the SCFMAX2 E3-ubiquitin ligase complex, which targets SMXL6/7/8 repressors for degradation. Notably, parallels between SL signaling and pathways involving auxin, gibberellin, jasmonate or salicylic acid suggest possible shared regulatory components. Inspired by recent discoveries of cyclic nucleotides (cAMP/cGMP) in auxin signaling, and based on bioinformatics and molecular modeling analyses, we identified putative adenylate/guanylate cyclase (AC/GC) motifs in D14, MAX2, and SMXLs. Recently, we tested whether D14, beyond its known hydrolase function, could also act as a guanylyl cyclase. Our *in vitro* assays show that D14 exhibits dual enzymatic activity: it hydrolyzes SLs upon ligand binding, and produces cGMP in the ligand-free state. Interestingly, calcium ions selectively enhance GC activity while inhibiting hydrolysis, suggesting a calcium-dependent regulatory switch. These findings support a novel model in which D14 integrates SL perception with cyclic nucleotide and calcium signaling, expanding our understanding of plant signal transduction complexity.

## additional info

This work was supported by “Excellence Initiative– Debuts” (ID – Debiuty) competition under “Excellence Initiative- Research University” program (IDUB).

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## Short- and long-distance communication in plants

### Oral presentation

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# How do plants manage their microelements? Zinc Translocation from Zn-Sufficient to Zn-Deficient Roots as an Adaptation to Heterogeneous Zn Availability

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Zinc is an essential element for plant development. However, its distribution in soil is heterogeneous, affecting e.g. roots growth efficiency. The control over Zn distribution in and between organs exists; however, the mechanisms that govern this process are unknown. We use transparent soil, a hydrogel-based medium that mimics soil properties and allows us to heterogeneously distribute Zn (Zn sufficient and deficient parts) and track root growth. We demonstrated that the expression of tobacco ZIPs (Zn-uptake), HMAs (Zn-translocation to shoot) and NASs (Zn-chelation) depends on the total accessibility of the root system to Zn, rather than local Zn levels. Further we confirmed, using  $\mu$ XRF at Polish Synchrotron SOLARIS, that under conditions of partial zinc deficiency in the medium (half with half without Zn), Zn is transferred between the Zn-sufficient and Zn-deficient lateral roots. This finding suggests the existence of unknown Zn homeostasis mechanisms, likely involving a complex process mediated by Zn transporters. These mechanisms would necessitate the unloading of Zn from the xylem and its subsequent loading into the phloem, a process supported by Zn status sensing and signaling to ensure precise execution within specific plant region. This work provides new insights potentially useful for future agricultural practices to address Zn deficiency.

## *additional info*

The research is realized with the funds of the National Science Center as part of the SONATA project (2020/39/D/NZ9/02393).

**Short- and long-distance communication in plants**

**Oral presentation**

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# *De novo* development of plant meristems

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The multicellular bodies of plants develop from single cells; the multicellular diploid phase of the plant life cycle develops from a polarised zygote, and the multicellular haploid phase develops from a spore without polarity. Each of these cell types divide to form masses of cells – enclosed embryos and free-living sporelings respectively. These cell masses form meristems, generative centres from which the mature body of the plant develops. The spore is produced by meiosis and lacks any markers of polarity. Upon germination, the spore polarizes *de novo*. This polarity orients the first cell division, which is asymmetric and produces a larger cell on apical side and a smaller cell on the basal side. The apical cell functions as a generative cell and divides while the basal cell terminally differentiates as a rhizoid cell. Preliminary data indicate that light polarises the spore cell and orients the first asymmetric cell division. A stem cell niche develops in cells derived from the apical cell and forms the plant body. Recent data on mechanism that operates during the development of cell polarity and the initiation of the meristem will be presented.

## *additional info*

This research is funded by the an ERC Advanced Grant and by a grant from the Austria Academy of Sciences.

**Early land plant biology**

**Keynote lecture**

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# Fast-evolving Liverworts: A Genomic Perspective from *Apoppelia endiviifolia* (Pelliales, Jungermaniopsida)

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The liverwort, *A. endiviifolia*, a dioecious and simple thalloid species, is notable for its cryptic diversity, habitat adaptability, genomic innovation, and basal phylogenetic position. These features make *A. endiviifolia* an essential model for exploring speciation mechanisms and evolution of genomic structures within liverworts. We present the genome assembly of haploid *A. endiviifolia*, with a total size of 2,914,960,273 bp and an N50 of 468,157,909 bp, demonstrating high completeness (99.2% BUSCO) and accuracy (QV 47.6). The assembly consisted of nine chromosomes, which included validated 18 telomeres and nine centromeres (ranging from 1.9 to 5 Mbp in length). RNA-seq-based annotation identified 34,615 genes, predominantly protein-coding. The TEs comprised 12.16% LTRs elements and 57 Helitrons. Among the retroelements, the Copia and Gypsy superfamilies comprised 8.94% and 2.95% of the genome, respectively. Ty3/Gypsy superfamily were found to be significantly enriched in centromeric regions. The average GC content ranged from 38.8% to 39.6%, with gene density varied between a value 5.52 and 9.78. Synteny analysis of related liverwort species revealed complex chromosomal relationships, indicating extensive genome rearrangement. Assembly and annotation offer valuable resources for investigating liverwort evolution, centromere biology, and genome expansion in simple thalloid liverworts.

## *additional info*

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## Early land plant biology

### Keynote lecture

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# Changes of biochemical composition in *Chlamydomonas reinhardtii* treated with brassinolide

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Various algae species present great potential in many industries, wastewater treatment, sequestering CO<sub>2</sub> from atmosphere, researches and biotechnology. Mostly they are displaced by higher plants due to their efficiency and easier growth. Usage of brassinosteroids (BRs), plant steroid hormones could make algae grow faster, less likely to get contaminated by bacteria or fungi, and more resistant to abiotic stress. Such addition of exogenous BRs can also influence biochemical composition of algal mass due to their impact on plant cells like increasing gene expression, growth, development, photosynthetic rate and other metabolic processes. *Chlamydomonas reinhardtii* is one of the species that present great potential in food, pharmaceutical or biofuel industries. That's why we conducted a research on its cell count and chosen biochemical parameters after the exposition to different concentration of brassinolide- one of the most active BRs. Results suggests, that exposition to proper brassinolide concentrations help in *C. reinhardtii* cultures and change concentrations of protein, monosaccharides and cell count. Such knowledge may be useful in future usage of different algae species and establishing proper BRs concentration for their growth in specific conditions changing the face of many industries.

**Early land plant biology**

**Oral presentation**

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# MpSPL3 gene is indispensable for generative organs development in *Marchantia polymorpha*

SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) genes encode plant-specific transcription factors that are widely distributed across the plant kingdom. In angiosperms, the multimember SPL family regulates different biological processes, such as vegetative-to-reproductive phase transition, inflorescence architecture, and lateral organ development. In contrast, the liverwort *Marchantia polymorpha* genome encodes only four SPLs, with functional studies available only for microRNA-targeted members, MpSPL1 and MpSPL2. While MpSPL1 was shown to control the meristem dormancy to modulate the thallus architecture, MpSPL2 was found to promote the transition from vegetative-to-reproductive phase. Here, we show that the MpSPL3 gene is crucial for successful coordination of the vegetative growth and the reproductive phase transition. The knockout of MpSPL3 leads to strong growth retardation with disordered thallus branching, reduced gemma cup number, and, most profoundly, abolished gametangiophores production. Additionally, MpSPL3 is responsible for direct or indirect expression control of other MpSPL family members and important genes for germ cell specification, like MpLRL (LOTUS JAPONICUS ROOTHAIRLESS-LIKE) and MpCKI1 (CYTOKININ-INDEPENDENT 1), since the expression level of these genes is significantly downregulated in the null *Mpspl3* background. Altogether, our findings indicate that MpSPL3 plays a crucial role in the regulation of gametophyte development and reproductive success in *Marchantia*.

## additional info

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## Early land plant biology

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# Study on liverwort-specific miRNAs – MpmiR11796 and MpmiR11887 involved in the sexual organ development of *Marchantia polymorpha*

miRNAs regulate nearly all developmental processes in plants. While conserved miRNAs are key indicators of land plant evolution and adaptation, the majority of non-conserved miRNAs in non-vascular plants have not been studied, raising questions about their functionality. Using *Marchantia polymorpha*, we investigated the expression pattern and functions of two liverwort-specific miRNAs. MpmiR11796 exhibited differential expression across vegetative and reproductive tissues, with high accumulation in archegoniophores. Genomic database and experimental analyses revealed that MpmiR11796 gene represents an independent intron-less transcriptional unit, although overlapping with a protein-coding gene. Promoter activity was observed predominantly in pegged rhizoids within digitate rays and stalk of archegoniophores. CRISPR-Cas9 male  $\Delta$ mpmir11796ko plants produced less rhizoids during gemmae development, while female  $\Delta$ mpmir11796ko plants showed reduction in archegonial receptacle size, stalk length, and abnormalities in egg cell development, thereby, affecting fertilization efficiency. MpmiR11887 showed accumulation exclusively in antheridiophores. Promoter activity was detected predominantly in antheridia and spermatogenous cells.  $\Delta$ mpmir11887ko plants showed early development of antheridiophores with larger antheridial discs and mature antheridia. Transcriptome and degradome analyses identified putative targets for both miRNAs. Our findings highlight regulatory roles of these liverwort-specific miRNAs in *Marchantia*'s sexual organ development and suggest their involvement in the regulatory networks unique to early land plants.

## additional info

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## Early land plant biology

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# MpmiR11889 controls sperm cell formation and boosts reproductive efficiency in *Marchantia polymorpha*

MicroRNAs are master regulators of gene expression, fine-tuning developmental processes across eukaryotic species. In *Marchantia polymorpha*, both conserved and liverwort-specific miRNAs guide key developmental transitions. Several miRNAs show distinct expression patterns in reproductive organs compared to vegetative thalli, underscoring their critical roles in reproductive development. Among these, the male-specific MpmiR11889 is especially compelling. Conserved across algae and liverworts, MpmiR11889 is exclusively expressed in *Marchantia* sperm cells. Its only validated target is the mRNA encoding MpDUSP12, a dual-specificity phosphatase evolutionarily conserved across eukaryotes. In mammals, DUSP12 regulates cell proliferation and differentiation; in fungi, it controls sporogenesis. MpDUSP12 contains a unique, conserved C-terminal zinc-finger domain, hinting at DNA-binding functionality. Our findings demonstrate that the MpmiR11889–MpDUSP12 module is essential for proper sperm development and reproductive success. Plants lacking MpmiR11889 ( $\Delta\text{mpmir11889}^{\text{9e}}$ ) or overexpressing MpDUSP12 ( $\text{MpDUSP12}^{\text{oe}}$ ) produced sperm with bead-like nuclei, elevated protamine-like protein (MpPRM), and abnormal flagellar structures. Transcriptomic and proteomic analyses revealed increased expression of genes and proteins associated with chromatin architecture, DNA repair, and cellular growth. These mutants exhibited severely disrupted sperm motility and chaotic movement, and crosses involving them showed dramatically reduced sporophyte formation (3–14% vs wild type). These findings highlight an ancient, conserved regulatory mechanism controlling spermatogenesis and gamete differentiation.

## additional info

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## Early land plant biology

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# Using AI to Predict Gene Regulation and Guide Experiments in Plants

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Gene regulation in plants is controlled by transcription factors (TFs) binding to specific DNA sequences called cis-regulatory elements (CREs). However, it's still difficult to predict how changes in these DNA regions affect gene activity, especially under different environmental conditions. In this study, we used a large collection of existing experimental data on TF-DNA binding in *Arabidopsis thaliana* to train an explainable AI model. The model learns how DNA sequence and context influence TF binding across the genome. Once trained, the model can predict TF binding in new genotypes, tissues, and conditions – essentially simulating experiments *in silico*. It reveals which DNA variants might disrupt or enhance TF binding, helps identify regulatory elements linked to traits, and suggests which genes may be affected. We tested the model on maize and showed it could detect TF binding responses to heat stress, even in this distant crop species. This approach creates a feedback loop: experimental data trains the model, the model generates new hypotheses, and these predictions guide the next experiments. It highlights how integrating AI with experimental biology can accelerate the discovery of gene regulation mechanisms and the functional impact of genetic variation.

**Early land plant biology**

**Oral presentation**

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# Poster abstracts

# BRM and MINU1/2 ATPases Orchestrate Organ-Specific RNA Processing in Arabidopsis

Understanding the regulation of alternative transcription in plants is essential for uncovering how they adapt to environmental changes. SWI/SNF-type ATP-dependent chromatin remodeling complexes (CRCs) play a key role in controlling gene expression and development. While BRM, a SWI2/SNF2-type ATPase, has been implicated in various developmental and stress-related processes, its role in organ-specific alternative transcription remains unclear. This study investigates the interplay between BRM and two additional SWI2/SNF2-type ATPases, MINU1 and MINU2, representing respectively BAS and MAS subclasses of SWI/SNFCRCs, in this regulatory mechanism. We generated Arabidopsis double mutants (*brm/mini1* and *brm/mini2*) to assess phenotypic and transcriptional outcomes. The double mutants largely mirrored the *brm* phenotype, reinforcing BRM's central developmental role. However, additional morphological traits indicated contributions from MINU1/2 subunits of MAS subclass of SWI/SNF. . To assess transcriptional regulation, we analyse the organ-specific use of transcription start sites (TSS) in the SEC14 gene. BRM inactivation led to altered TSS selection in an organ-specific manner. The combined loss of MINU1/2 in the *brm* background modified these patterns further, suggesting a synergistic function of BRM- and MINU-containing subclasses of CRCs. Our findings highlight a cooperative role of SWI/SNF CRCs subclasses in alternative transcription control, with implications for plant development and adaptation strategies.

## *additional info*

The research was carried out as part of the project SONATA National Science Centre 2021/43/D/NZ2/02461.

**DNA and RNA metabolism,  
epigenetics and genomics**

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# Development of a Cross-Species VHH-Based Platform for Discovering Drugs Against Metabolism-Related Human Diseases

Tumours often exhibit disruptions in key regulatory processes, including chromatin remodelling by SWI/SNF complexes and mRNA splicing, which affect metabolism and gene expression. In clear cell renal cell carcinoma (ccRCC), TOR (Target of Rapamycin) hyperactivation and metabolic reprogramming are common, and TOR inhibitors are used clinically. Using *Arabidopsis thaliana* and human cell lines (HeLa, A498), we explored conserved mechanisms of gene regulation and developed a VHH-based platform for targeted protein inactivation. We found that nuclear mTOR interacts with the NineTeen Complex (NTC), involved in RNA splicing, and regulates phosphorylation of both NTC and SWI/SNF subunits. We also discovered that TOR and SWI/SNF physically interact and co-bind the promoter of FBP1, a key metabolic gene. Loss of SWI/SNF function leads to TOR hyperactivation. Everolimus, an mTOR inhibitor, had minimal transcriptomic impact in wild-type *Arabidopsis* but triggered broad changes in the *swi3c* mutant. This enabled development of a mutant-based screening system to identify TOR-related compounds. Additionally, we are isolating VHHs targeting TOR's catalytic domain. Our findings reveal a conserved regulatory network linking chromatin remodelling, RNA splicing, and metabolism, offering insights into ccRCC and new avenues for cancer therapy.

## additional info

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**DNA and RNA metabolism, epigenetics and genomics**

## Poster 2

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# Does the role of nitrilases in the embryogenic transition process involve the synthesis of PAA?

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The NITRILASE 1 subfamily evolved through gene duplication from an ancestral NIT4, and comprises: NIT1–NIT3. Previous studies indicate the involvement of NIT1–NIT3 in somatic embryogenesis (SE), although the mechanism of their action during the embryogenic transition remains unknown. Literature data suggest that NIT1–NIT3 are involved in the biosynthesis of indoleacetic acid (IAA) and phenylacetic acid (PAA). However, nit mutants show no significant differences in IAA content, leading to the hypothesis that NITs are involved in PAA synthesis. PAA is synthesized from the phenylalanine precursor via the CYP79A2-dependent phenylacetaldoxime pathway, which is further converted into phenylacetonitrile/benzyl cyanide (PAN/BnCN). PAN/BnCN serves as a substrate for NIT1–NIT3, which convert it into PAA. Studies have shown that treating explants with PAN/BnCN induces SE process. Transcriptome analysis revealed that treatment with PAN/BnCN activates a number of genes with confirmed and documented roles in the SE process, including SE master regulators. Additionally, an increased auxin response following PAN/BnCN treatment was confirmed using DR5::GUS reporter lines. The inhibition of NIT1–3 activity by heatin negatively affects the ability of explants to undergo SE under PAN/BnCN treatment. These findings suggest that the role of NIT1–3 in SE process is potentially related to their function in PAA biosynthesis.

## *additional info*

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**DNA and RNA metabolism, epigenetics and genomics**

**Poster 3**

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# Genetic basis of transcriptomic diversity in maize

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Genetic variation that influences gene expression plays a fundamental role in shaping phenotypic diversity. However, despite its significance, large-scale studies of transcriptome diversity in plants have remained limited. In this study, we analyzed gene expression profiles from 631 maize inbred lines using leaf tissue collected during a field experiment. We found that the vast majority of gene expression variation (95%) was distributed within maize heterotic groups (subpopulations), reflecting underlying DNA sequence diversity. Furthermore, we mapped genetic variants associated with gene expression – cis-expression quantitative trait loci (cis-eQTLs) – and identified over than 64,000 putatively causal eQTLs. A substantial proportion of genes (78.6%) harbored two or more independent eQTLs, revealing extensive allelic heterogeneity in the genetic regulation of gene expression in maize. Finally, we observed that evolutionarily constrained genes tend to have fewer eQTLs with smaller effect sizes, suggesting presence of weak purifying selection against expression-altering variants. Together, our study provides new insights into the diversity and regulation of gene expression in maize.

**DNA and RNA metabolism,  
epigenetics and genomics**

**Poster 4**

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# Assessment of Cas12a Ribonucleoprotein Complex Activity *in vitro* for Future Use in Plant Genome Editing

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The CRISPR-Cas system is a revolutionary genome editing technology that has transformed molecular biology. Its precision and versatility enable targeted genetic modifications across a wide range of organisms. In plant research, DNA editing can be achieved by introducing ribonucleoprotein (RNP) complexes into explants or protoplasts, allowing accurate modifications without the integration of foreign DNA. We have previously demonstrated that this system is applicable to the editing of the flavanone 3-hydroxylase (F3H) gene in carrots (*Daucus carota*). This study aimed to evaluate the efficiency of DNA cleavage by Cas12a RNP complexes under varying conditions, including temperature, incubation time, and storage duration. The Cas12a protein used was derived from *Acidaminococcus* sp. BV3L6. All experiments were conducted *in vitro* using FAM-labelled, PCR-amplified F3H gene fragments. To assess the influence of temperature, reactions were carried out at 22°C to 37°C. Digestion times ranged from 15 minutes to 24 hours. Additionally, the stability of preassembled RNP complexes was evaluated after storage periods ranging from one to seven days. The results revealed the optimal conditions for DNA cleavage and highlighted the limitations of using Cas12a RNP complexes. These optimized parameters will be applied in future experiments focused on genome editing in plant protoplasts.

## *additional info*

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DNA and RNA metabolism,  
epigenetics and genomics

Poster 5

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# Plant homologs of human splicing factors contribute to a stress-responsive complex in *Arabidopsis*

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Sessile in nature, plants must rapidly and globally reprogram gene expression in response to environmental stimuli. This reprogramming encompasses both transcriptional regulation and alternative splicing. We characterized a previously unreported protein complex in *Arabidopsis thaliana*, comprising six subunits, with its core component identified as the homolog of the human spliceosomal factor CWC22. Another constituent of the complex is an ortholog of CWC21/SRRM2, a key human splicing regulator. Additional components include splicing regulators SR34a and SC35, as well as two cyclophilin-like peptidyl-prolyl cis-trans isomerases CYP95 and CYP63. Protein-protein interactions among these subunits were validated through both *in vivo* and *in vitro* assays. Furthermore, FRAP experiments revealed that CWC22 forms dynamic nuclear condensates. As human CWC22 interacts with EIF4A3, we investigated the *Arabidopsis* homolog and discovered novel interactions between EIF4A3 and both CWC22 and CWC21. Functional analysis under salinity stress demonstrated that *A. thaliana* insertion mutants *cwc22*(+/-), *cwc21*(-/-) and *cyp95*(-/-) exhibit significantly enhanced germination rates. Transcriptomic profiling via RNA-seq confirmed that this complex modulates gene expression and alternative splicing in response to salinity stress. These findings reveal a novel splicing-associated complex in *Arabidopsis*, integral to transcriptomic plasticity and adaptive responses to abiotic stress, highlighting its potential role in enhancing plant resilience.

DNA and RNA metabolism,  
epigenetics and genomics

Poster 6

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# Can the E179A Mutation and His-Tag Fusion Site Affect the Activity of *Arabidopsis thaliana* FEN1?

FEN1 (Flap Endonuclease 1) is a structure-specific endonuclease involved in DNA replication and repair. It exhibits three distinct: FEN, gap-dependent endonuclease (GEN), and nick-specific exonuclease (EXO) enzymatic activities. Human FEN1 protein with E178A mutation retains only the first one (i.e. FEN), suggesting that these three activities may be separable. In this study, we analyzed the effect of the E179A mutation on the enzymatic activity and aggregation of *Arabidopsis thaliana* (At) FEN1, which even in its native form is unstable and easily undergoes precipitation. For this purpose, an expression vector encoding the AtFEN1 E179A mutein was prepared using the Gateway system. The recombinant protein was produced in *Escherichia coli* and purified *via* affinity chromatography. It was then subjected to *in vitro* enzymatic activity assays using specifically designed DNA substrates. Our results show that the E179A mutation significantly reduces the FEN activity of AtFEN1 and increases its propensity to aggregate. Previous studies suggest that FEN1 protein retained its normal activity only when fused to a His-tag at the C-terminus, but not at the N-terminus. To test this hypothesis, we utilized wild-type AtFEN1 tagged at either N- or C-termini and supported our investigation with bioinformatic analysis.

## *additional info*

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**DNA and RNA metabolism,  
epigenetics and genomics**

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# Functional crosstalk between chromatin remodeling, m6A methylation, and alternative RNA processing in *Arabidopsis*

In eukaryotes, gene expression regulation requires a coordinated interplay between chromatin structure, transcription, and post-transcriptional mRNA processing. Our work uncovers a functional crosstalk between SWI/SNF chromatin remodeling, m6A RNA methylation, and alternative RNA processing in *Arabidopsis thaliana*. We demonstrate that the SWI3C subunit of the SWI/SNF complex interacts with CDC5, a core component of the spliceosome activating complex (NTC), and with MTA, the catalytic subunit of the m6A writer complex. Through co-immunoprecipitation, Y2H, BiFC assays, and transcriptomic analyses, we show that these proteins form a molecular hub regulating alternative transcription. Notably, loss-of-function mutants in SWI3C and CDC5 exhibit reduced global m6A levels and altered chromatin states at stress-responsive loci, such as PLATZ. Structural predictions suggest that CDC5 acts as a molecular bridge via its HTH domain, enabling interaction between SWI3C and the MTA. Our results provide compelling evidence of an integrated mechanism whereby chromatin state and RNA modifications converge to shape transcript diversity. These findings propose a novel regulatory paradigm with broad implications for developmental plasticity and environmental adaptation in plants.

## additional info

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**DNA and RNA metabolism, epigenetics and genomics**

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# Cajal bodies and global poly(A)RNA retention: a new perspective on nuclear dynamics in plant cells

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Cajal bodies (CBs) are dynamic nuclear structures involved in the maturation and modification of RNA as well as in the biogenesis and regulation of spliceosomal components. While their function has been extensively characterized in animal cells, the roles of CBs in plant cells remain less understood. In this study, we present novel insights into the involvement of Cajal bodies in nuclear mRNA retention and the regulation of spliceosomal machinery availability in plant cells exhibiting pulsatile transcriptional activity. Using high-resolution imaging techniques, including confocal and STED microscopy, we demonstrate that CBs dynamically alter their composition – in terms of both RNA and protein content – between successive transcriptional pulses. This suggests that CBs function in an adaptive manner, modulating their molecular content in response to the cell's current transcriptional state. Our findings indicate that Cajal bodies may act as specialized hubs coordinating the maturation of mRNA and the spatial-temporal availability of spliceosomal components. We propose that, beyond their canonical role in RNP biogenesis, CBs serve as regulatory centers governing the timing of mRNA release for translation, thereby contributing to the fine-tuned control of gene expression in plant nuclei.

## *additional info*

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**DNA and RNA metabolism, epigenetics and genomics**

**Poster 9**

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# VAL transcription factors with the PRC1 and PRC2 complexes regulate The somatic embryogenesis in Arabidopsis

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The unique structural organization of the male germ unit (MGU), consisting of two sperm cells and the vegetative cell nucleus, and the female (FGU) germ unit, comprising the synergids, egg cell and central cell, is critical for successful sexual reproduction in *Arabidopsis thaliana*. We identified specialized cellular connections that facilitate communication within these reproductive units using light and electron microscopy. In the FGU, we observed potential symplasmic connections between egg and central cells, with an irregular and discontinuous extracellular matrix between the cells that brings their plasma membranes into direct contact at specific regions. Through multiple staining approaches and immunocytochemistry, we demonstrate that the extracellular matrix surrounding the FGU cells has a unique composition distinct from the typical plant cell wall. Within the MGU, we documented a physical association between one sperm cell and the vegetative nucleus, where the sperm cell's cytoplasmic projection becomes enveloped by the lobed structure of the vegetative nucleus. This association enables coordinated movement of sperm cells within the growing pollen tube but also facilitates direct communication between MGU cells. These physical connections between cells in MGU and FGU, provide structural routes for potential symplastic transport and cell-cell communication, underlying documented molecular exchange between these cells.

**DNA and RNA metabolism, epigenetics and genomics**

**Poster 10**

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# The draft genome assembly and annotation of allotetraploid *Festuca glaucescens*

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**Background:** We present the first long-read genome assembly and annotation for *Festuca glaucescens*, a member of a genus characterized by polyploidy, large genome sizes, and high repeat content. Fescues are important forage and turf grasses in temperate regions, valued for their resilience to abiotic stress. The allotetraploid *F. glaucescens* is particularly notable for its drought tolerance, yet its genomic architecture has remained largely unexplored. **Results:** The haploid assembly spans 5.52 Gb, with >98% gene space completeness as assessed by BUSCO. Repetitive elements comprise ~77% of the genome, dominated by Gypsy and Copia LTR retrotransposons. We predicted 72,385 protein-coding genes, the majority of which are supported by transcriptomic and homology-based evidence. While some contig-level fragmentation persists, the assembly successfully captures the gene-rich, repeat-dense landscape of this complex polyploid genome. **Conclusions:** This is the first annotated reference genome for a *Festuca* species, offering a foundational resource for functional genomics, comparative studies, and forage grass improvement.

*additional info*

**Keywords:** *Festuca glaucescens*, genome assembly, polyploidy, repeatome, gene annotation, functional genomics, forage grasses

National Science Centre, Poland (project no. 2020/39/B/NZ9/02488).

**DNA and RNA metabolism, epigenetics and genomics**

**Poster 11**

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# Sequencing and assembling three non-reference *Medicago truncatula* genomes with long reads

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Symbiotic nitrogen fixation in legumes occurs in root nodules, which develop after mutual recognition between the host plant and colonizing symbiotic rhizobia. The nodulation process in *Medicago truncatula* is genetically programmed and involves significant shifts in gene expression, particularly the activation of gene clusters within the so-called “symbiotic islands”. Additionally, numerous transposable elements (TEs) adjacent to the activated genes undergo transient activation at the early stages of nodule development, but become hypermethylated and silenced in mature nodules. These observations led us to explore whether natural variation in TE copy number and genomic positioning could influence the epigenetic landscape and expression of symbiosis-related genes. However, due to limitations in the resolution of short-read sequencing data generated within the HapMap project, insights into structural variation across *M. truncatula* accessions have remained limited. Moreover, high-quality, chromosome-scale genome assemblies are currently available for only three accessions. To overcome these limitations, we generated chromosome-level assemblies for three additional geographically distinct *M. truncatula* accessions using a combination of PacBio and Oxford Nanopore long-read technologies. We subsequently performed de novo annotation of both genes and TEs in these new genomes. The findings from this comparative analysis will be presented and discussed.

## additional info

This work was carried out as part of my doctoral research funded by the PRELUDIUM BIS 3 grant (2021/43/O/NZ2/01626) and an international internship supported by the PRELUDIUM BIS NAWA 3 (BPN/PRE/2023/1/00023).

**DNA and RNA metabolism, epigenetics and genomics**

**Poster 12**

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# Three-dimensional 35S rDNA arrangement in the nucleoli of monocot and eudicot representatives

The nucleolus is one of the most prominent structures within eukaryotic cells, primarily due to its essential role in 35–48S ribosomal RNA (35S–45S rRNA in plants) synthesis and ribosomal subunit biogenesis. However, our current understanding of the structure, function, and dynamics of plant nucleoli remains limited, particularly compared to human, animal, and yeast nucleoli. Both plant and animal nucleoli typically comprise three principal subregions: the fibrillar centre, dense fibrillar component, and granular component. Notably, most plant nucleoli contain a distinct structure known as the nucleolar vacuole, the functions and variability of which are still poorly understood. Previous studies have largely relied on ultrastructural analyses using transmission electron microscopy, providing only a fragmentary, two-dimensional perspective of nucleolar architecture, especially in the context of the spatial arrangement of 35–45S ribosomal DNA (rDNA) loci. Thus, three-dimensional visualisation is crucial for advancing our understanding of nucleolar organisation in plants. In this study, we employed state-of-the-art laser-scanning confocal microscopy to visualise the arrangement of 35S rDNA in prophase I meiocytes and interphase nuclei isolated from somatic cells of *Brachypodium* sp. and *Nicotiana benthamiana* Domin., thereby providing new insights into the spatial dynamics of plant nucleoli.

## additional info

This work was supported by funding for the scientific activities of doctoral students under Competition No. 17 and 18, provided by the Doctoral School of the University of Silesia in Katowice, and by the National Science Centre Poland, under the grant no. 2018/31/B/NZ3/01761.

**DNA and RNA metabolism, epigenetics and genomics**

**Poster 13**

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# Epigenetic Regulation of Premature Leaf Senescence in Barley: Dynamic Interplay Between Histone Modifications and DNA Methylation

Recent investigations have revealed the involvement of histone modification in regulating leaf senescence; however, the molecular mechanisms underlying the onset and progression of histone modification-mediated leaf senescence remain poorly understood. In this study, we investigated the epigenetic landscape of dark-induced leaf senescence (DILS) in barley, focusing on two histone modifications: H3K9ac (an activation mark) and H3K9me2 (a repression mark). Genome-wide ChIP analysis across 4, 7, and 10 days of darkness revealed dynamic redistribution of both marks. H3K9ac was enriched at genes involved in autophagy, stress response, RNA processing, and hormonal regulation. Conversely, H3K9me2 accumulated at loci related to chromatin silencing, cell cycle arrest, and DNA methylation pathways. Venn diagram analyses indicated a temporally distinct and only partially overlapping set of genes marked by H3K9ac or H3K9me2, suggesting a sequential and coordinated switch between transcriptional activation and repression. Functional enrichment highlighted the role of chromatin remodelling in orchestrating transitions under stress. Our findings demonstrate that histone acetylation and methylation act as key regulators of transcriptional reprogramming during premature leaf senescence and offer a framework for modulating senescence in crops.

## *additional info*

This work was supported by the National Science Centre, Poland, under the grant no. 2018/30/E/NZ9/00827 to ES-N.

**DNA and RNA metabolism, epigenetics and genomics**

**Poster 14**

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# Uncovering genes under MONOPTEROS regulation during the embryogenic transition in *Arabidopsis thaliana*

The process of somatic embryogenesis (SE), mainly induced by auxin, leads to the formation of bipolar structures called somatic embryos. Studies on the process of *Arabidopsis thaliana* SE contribute to identifying key components that turn on the embryogenic developmental pathway, i.e., transcription factors, miRNAs, or epigenetic markers. One of the genes playing a key role in the SE is the MONOPTEROS (MP) gene. MP undergoes the strongest expression during the *Arabidopsis thaliana* SE process. Additionally, the MP isoform, called MP11ir, was identified as being involved in SE. The MP11ir transcript, an alternatively spliced variant of MP, produces a truncated protein missing the PB domain, crucial for repression of MP by Aux/IAAs. Both MP and MP11ir are essential for embryo regeneration in the mp mutant. However, overexpressing truncated MP protein ( $\Delta$ ARF5) lacking the PB1 domain inhibits SE, leading to callus formation instead of somatic embryos. The precise mechanism of MP and MP11ir action in SE is unknown. Transcriptome analysis was done in different transgenic lines to learn about the MP and MP11ir mechanism of action. The study identified a set of genes potentially under MP and/or MP11ir control.

## additional info

This research was funded in part by the National Science Centre, Poland, under the OPUS call in the Weave programme (2023/51/I/NZ1/00704).

**DNA and RNA metabolism, epigenetics and genomics**

**Poster 15**

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# Decoding plant gene regulation with deep learning from omics data

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The activity of cis-regulatory elements (CREs), key drivers of gene expression, is governed by complex interactions between DNA sequence and regulatory proteins. We address the challenge of modeling this complexity by developing deep learning approaches trained with transcriptomic and DNA-protein interaction (OMICS) data. These models learn intricate sequence-function relationships, allowing for accurate prediction of CRE activity, identification of critical regulatory features, and ultimately, a better understanding of how CRE variation influences gene expression.

**DNA and RNA metabolism, epigenetics and genomics**

**Poster 16**

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# Amaryllidaceae alkaloid profiling in different *in vitro* cultures of *Narcissus poeticus* var. *recurvus* (Haw.) Baker

The Amaryllidaceae family is a source of pharmacologically active alkaloids, especially galanthamine and lycorine. Galanthamine, used in Alzheimer's treatment, is traditionally obtained through chemical synthesis or plant extraction – both economically inefficient. Lycorine is undergoing clinical trials as a potential anticancer agent. Currently, alternative methods of obtaining these alkaloids through *in vitro* cultures are being investigated. For the first time in this study, cultures of embryogenic callus, somatic embryos, adventitious roots, adventitious bulbs, and plants were established from *Narcissus poeticus* var. *recurvus* (Haw.) Baker, and their biosynthetic capacity was evaluated. GC–MS analysis revealed the presence of seven alkaloids in the obtained plant materials: galanthamine, 1,2-didehydrocrinan-3-ol, anhydrolycorine, crinine, cheryline, 11-hydroxyvittatine, lycorine and tyramine compounds involved in the biosynthesis pathway of alkaloids. However, alkaloid diversity depended on tissue or organ differentiation. The highest alkaloid diversity was observed in plants (six alkaloids); the lowest was found in embryogenic callus, somatic embryos, and roots (two alkaloids in each culture). Crinine had the highest percentage contribution to the extract, comprising 30.10%–91.95% of total ion current. LC–MS analysis showed the highest galanthamine concentration (2.67 µg/g dry weight, DW) occurred in bulbs, while the greatest lycorine content (33.5 µg/g DW) was detected in plants.

**Proteomics, metabolomics and phenomics**

**Poster 17**

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# Searching for new mechanisms of maize adaptation to cool spring conditions

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Despite years of adaptation to the temperate climate, the growth of maize could be restricted by cold at the early stages of development, when the photosynthetic apparatus is forming. The experiments used seedlings of four maize inbred lines: A554, S018693, S311, and S84854. According to previous extensive phenomic studies on a wide set of genotypes (Sowiński et al., 2025, doi.org/10.1186/s12870-025-06198-2), A554 and S018693 cultivated in cold exhibited rapid growth despite poor physiological status. In contrast, S311 and S84854 exhibited slower growth but better physiological status. To study the physiological and molecular mechanisms underlying the observed differences, we examined those lines for photosynthetic apparatus status using the OJIP test and quantified gene expression using RNA-seq. All procedures were performed at the V1 growth stage for the control plants (24°C/22°C), the cold-grown plants (16°C/12°C) and during several consecutive days of regrowth (24°C/22°C). In the experiments two parts of plants were used, the developing second leaf (OJIP, RNA-seq) and shoot apex (RNA-seq). The results showed different ability of studied lines to recover photosynthetic apparatus after seedling development in cold. This was accompanied by both line-specific and non-specific gene expression changes. Among the notable line-specific gene groups, the circadian rhythm is particularly noteworthy.

## *additional info*

This work was supported by Grant 2020/39/B/NZ9/00801 from the National Science Centre (NCN), Poland.

**Proteomics, metabolomics and phenomics**

**Poster 18**

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# Genetic insights into microspore embryogenesis induction in wheat

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Isolated microspore culture is the most effective method of doubled haploid production which are widely utilized in breeding and basic research. After pre-treatment with stress factor, microspores which *in vivo* develop into mature pollen grains are reprogrammed to follow a sporophytic pathway of development, leading to the development of androgenic embryos. Numerous factors affecting the effectiveness of androgenesis including the type and duration of pre-treatment, culture conditions, stage of the microspores at the initiation of *in vitro* culture. However, androgenesis remains a highly genotype-dependent process, and even optimized protocols often fail in certain genotypes. The molecular mechanisms and specific genes underlying this genotype dependency remain largely unknown. In this study, we analyzed gene expression during both *in vivo* microspore development and *in vitro* androgenic induction. The objective was to identify genes whose expression correlates with androgenic potential. The analysis was performed in two spring and two winter genotypes of *Triticum aestivum* L. differing in their responsiveness to isolated microspore culture. We believe this approach will enable the identification of genes that can serve as a marker of effective induction of androgenesis. Additionally, this work contributes to the development of more broadly applicable protocols for DH production in wheat breeding programs.

## additional info

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**Application of Plant Biotechnology for Sustainable Agriculture**

**Poster 19**

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# Effects of various cytokinins on some key metabolic enzymes and chloroplasts in tissue cultures of McIntosh apple scion

Apple (*Malus x domestica* Borkh.) is one of the most harvested fruit crop in the world. In our experiments thidiazuron (TDZ), 6-benzylaminopurine riboside (BAR) and meta-topolin (TOP) were used as cytokinins in plant tissue culture of apple shoots. Lysosomes and vacuoles containing inorganic phosphate are rich in acidic phosphatases. Alkaline phosphatase activity can be detected in the cytoplasm and chloroplasts of a plant cell. Together with acidic phosphatase, these are the non-specific phosphatases of plants. Triose phosphatases are important enzyme intermediates in photosynthesis, glycolysis, respiration and important in maintaining energy supply of cells under stress.  $\beta$ -NAD diphosphatase plays important role in energy transport between chloroplasts and mitochondria. Peroxidases and polyphenol oxidases take part in eliminating of ROS in plant cells. FRAP indicates the antioxidant capacity of plant tissues. The effect of TDZ, BAR and TOP on the ultrastructure of chloroplasts was studied with transmission electron microscopy. The effect of TDZ and BAR was similar on acidic phosphatase activity while alkaline phosphatase activities were comparable in TDZ-, BAR- and TOP-treated plants. Highest triose phosphatase,  $\beta$ -NAD diphosphatase and polyphenol oxidase activities were determined in TOP-treated plants. Furthermore, amount of amyloids and structure of thylakoid membranes were different as a result of TOP treatment.

## additional info

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## Application of Plant Biotechnology for Sustainable Agriculture

### Poster 20

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# Gynogenesis in tomato (*Solanum lycopersicum* L.) and transcriptomic analysis of callus derived from ovule explants

Biotechnological strategies such as plant haploidization offer promising tools to accelerate crop improvement. A major advantage is the rapid production of true homozygous lines through genome doubling of haploids, yielding doubled haploids (DH). While effective in many crops, haploidization in tomato remains challenging. Early androgenesis attempts via anther and microspore cultures resulted only in limited callus formation without regeneration. Gynogenesis led to plant regeneration but primarily from somatic tissues. This study aimed to induce haploid development from the female gametophyte using two male-sterile breeding lines (II-PS and I-MS10) and two fertile cultivars ('Progress F1' and 'Mieszko F1'). Ovaries were collected at anthesis, with fertile lines emasculated earlier to prevent self-pollination. Ovules were aseptically isolated and cultured on B5 medium with TDZ, NAA, and 2,4-D. Callus formation occurred in all genotypes, with responses varying by genetic background. Flow cytometry revealed haploid, diploid, and mixoploid callus. Three callus types – embryogenic green (EG), embryogenic white (EW), and non-embryogenic (SNE) – were selected for transcriptomic profiling. RNA-seq, performed on male-sterile lines, revealed significant gene expression differences between embryogenic and non-embryogenic calluses, including upregulation of genes related to oxidative stress response, polysaccharide metabolism, and cell wall remodeling.

**Application of Plant Biotechnology for Sustainable Agriculture**

**Poster 21**

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# Somatic embryogenesis and antioxidant compound contents in callus of three colored varieties of carrot (*Daucus carota* L.)

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Carrots are a source of bioactive compounds – carotenoids, anthocyanins (in purple varieties) and polyphenols. These compounds have antioxidant properties, playing an important role in the prevention of lifestyle diseases. Therefore, there is a growing demand for varieties with a high content of these substances. The aim of the study was to establish procedures for obtaining callus and somatic embryos of colored carrot varieties. The effect of cultivation on the content of antioxidant compounds was also examined, which is a starting point for further work on modifying their content. From 10 varieties (yellow, orange, and purple), these with the highest callus formation capacity (Juane du Doubus, Chantaney, Deep Purple) were selected for further research. The callus was transferred to different media to obtain somatic embryos. The content of antioxidant compounds was determined in the callus extracts by spectrophotometric methods, and the antioxidant activity was measured by the DPPH test. All varieties have both a clear embryogenic potential, especially the Deep Purple variety, and high antioxidant activity. Under the conditions of the experiment, this activity is primarily associated with the presence of phenolic compounds. This may enable the production of new varieties with increased content of bioactive compounds of various classes.

**Application of Plant Biotechnology for Sustainable Agriculture**

**Poster 22**

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# Developing *in vitro* systems as tool for crop improvement in garlic

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Garlic cultivars do not produce seeds and are propagated vegetatively. Optimizing efficient protocols for *in vitro* cultures, including protoplasts, opens the door to other applications of garlic improvement, such as cell electrofusion or vector-free transfection. These techniques generate variability more rapidly compared to conventional breeding methods. Although considerable progress has been made in this area, garlic is still considered recalcitrant to protoplast cultures. The objective of the study was to optimize factors involved in the production of garlic embryogenic callus, used as source material for protoplast isolation, and establish a reproducible protocol for plant regeneration in protoplast cultures. Embryogenic callus allows for plant regeneration. Callus-derived protoplasts were released using different compositions of maceration mixtures, with yield varying from 0.2-1.2 million cells per gram of fresh tissue. Released protoplasts were characterized by high viability (over 80%). An increase in size, cell wall reconstruction, and cytoplasm reorganization were observed in the early stages of culture. Usage of medium supplemented with SAHA, an inhibitor of histone deacetylation, led to suppression of division latency. Mitotic divisions were observed, resulting in cell colony development and microcallus formation. Proliferated callus formed proembryogenic mass and somatic embryos, that converted into plants, later acclimatized to *ex vitro* conditions.

## *additional info*

This work was supported by the grants of the Polish Ministry of Agriculture and Rural Development is acknowledged (grant no. KS.zb.802.12.2021 and DHR.hn.802.13.2022).

## Application of Plant Biotechnology for Sustainable Agriculture

### Poster 23

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# Development of molecular markers of resistance genes of blackcurrant (*Ribes nigrum* L.) to the pathogen *C. ribicola*

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White pine blister rust (WPBR) is one of the most dangerous diseases of blackcurrant. White pine blister rust is caused by *Cronartium ribicola*, a macrocyclic fungus that requires two different host plants – five-needle pines and plants of the *Ribes* genus, mainly blackcurrants to complete its complex life cycle. The genetic resistance of blackcurrant plants to white pine rust is not fully understood. For many years, this resistance (so-called monogenic resistance) was controlled by one dominant Cr gene, originating from the wild species *Ribes ussuriense*. It is now known that the resistance of currant plants conditioned by the Cr gene has been broken. The aim of this study was to search for molecular markers of resistance genes of blackcurrant to *C. ribicola* using RAPD- and SSR-PCR techniques. The study was conducted on 10 resistant and 10 susceptible genotypes blackcurrant to WPBR. The genotypes were selected based on the evaluation conducted under conditions of natural and artificial infection of the pathogen. PCR reactions were performed with 30 RAPD primers and 30 SSR primer pairs. Products differentiating resistant and susceptible genotypes were obtained for two RAPD primers and one SSR primer pair.

## *additional info*

This research was funded by the Ministry of Agriculture and Rural Development as part of basic research for Biological Progress in Plant Production, Tasks 44.

**Application of Plant Biotechnology  
for Sustainable Agriculture**

**Poster 24**

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# Evaluating the Vulnerability of Ukrainian-Bred, Cold-Hardy Table Grapes to *Drosophila suzukii* Colonization

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The invasive fruit fly *Drosophila suzukii* poses a significant threat to grape production due to its ability to oviposit in ripening, intact fruit. Ukrainian breeding programs have developed cold-hardy table grape cultivars with large berries and complex resistance to biotic and abiotic stressors. However, their susceptibility to *D. suzukii* remains largely unstudied. Our work evaluates the vulnerability of 11 such cultivars, bred by Anatolii Bochinsky, to *D. suzukii* infestation under controlled laboratory conditions. The study used a standardized infestation model involving 4 females and 4 males per berry, with oviposition monitored over 120 hours. Berries were later incubated to allow larval development, and infestation was quantified across multiple developmental stages. Parallel assessments included sugar content and behavioral observations during oviposition. Significant differences were observed in infestation intensity among cultivars, with some showing resistance manifested as low larval counts, absence of juice exudation upon puncture, and intact berry skin. Others supported high larval densities, likely due to favorable textural and biochemical traits. These findings highlight the potential of certain cold-hardy cultivars to resist *D. suzukii* and provide a foundation for targeted breeding efforts and integrated pest management strategies adapted to temperate viticulture.

**Application of Plant Biotechnology for Sustainable Agriculture**

**Poster 25**

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# *In silico* analysis of two uncharacterized Arabidopsis proteins, GERALT and TATOOINE, from the cryptochrome/photolyase family

The aim of our work was to analyze *in silico* proteins from the cryptochrome/photolyase family (CPF): GERALT and TATOOINE encoded by At2g47590 and At4g25290, respectively. Phylogenetic analysis indicates that GERALT belongs to the subclade sister of CRY-DASH, TATOOINE to the sister subclade of cyclobutane dimer-specific photolyases. According to the structural model of GERALT, its FAD-binding domain is shorter than that of other CPF proteins, so its mode of action is probably different from that described for cryptochrome/photolyases. The results of modeling the interaction of GERALT with potential partners indicate that it may be a logic gate, connecting light signals and the redox state of the chloroplast stroma, which is crucial at the beginning of plant growth. The phenotype of the *geralt* mutant - albino cotyledons, light green true leaves and impaired photosynthesis confirms this possibility. The presence, in addition to the photolyase and FAD-binding domains, of the lipase domain in TATOOINE indicates that it does not function as a typical photolyase/cryptochrome. What is the role of FAD in the regulation of TATOOINE activity, what is the substrate for the lipase domain and what is the physiological role of this protein are just a few questions that need to be answered.

## *additional info*

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**Biology of chloroplasts and plant mitochondria**

**Poster 26**

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# Prolamellar bodies as polycrystalline cubic membranes: orientation analysis through 3D modeling

The prolamellar body (PLB) is a paracrystalline membrane network that forms in etioplasts during plant growth in darkness and reorganizes into thylakoids upon illumination. Its architecture is based on a Schwarz D-type minimal surface, but unlike canonical cubic membranes the PLB exhibits an imbalanced topology with unequal aqueous channel volumes. In high-resolution TEM images, PLBs often appear as mosaics of multiple crystalline domains rather than as continuous, perfectly ordered lattices. To analyze this polycrystalline organization, we developed a custom computational framework that extends beyond existing tools. Our approach enables simultaneous modeling of multiple domains of the PLB network, simulation of 2D projections comparable to TEM images, and reconstruction of 3D polycrystalline models. The relative orientation of adjacent domains is then quantified by calculating the angle and relative position between their orientation vectors, providing a direct measure of grain boundary geometry within the PLB. This new methodology reveals that PLBs are not uniform cubic crystals but consist of distinct crystalline grains with well-defined angular relationships.

## *additional info*

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**Biology of chloroplasts and plant mitochondria**

**Poster 27**

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# Excessive light leads to RNA oxidation in the leaves of *Arabidopsis* (*Arabidopsis thaliana* L.) plants

Exposure to elevated light is a common stress factor, affecting the performance of plants. We propose a hypothesis that high light conditions cause disturbances in the process of photosynthesis leading to the over-production of reactive oxygen species (ROS) and associated RNA oxidation. Within the research 28-days old *Arabidopsis* plants were exposed for 1 and 5 hours to light of 120 (control), 800 and 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . High light stress disturbed the photosynthesis as reflected by decrease in maximum quantum yield ( $Q_y \text{ max}$ ) and in the ratio of fluorescence decrease (Fd) to steady-state fluorescence (Fs). This was accompanied by an increase in non-photochemical quenching (NPQ). On the other hand, excessive light had no effect on the level of chlorophyll. The disturbances in the process of photosynthesis were accompanied by intensified RNA oxidation, as reflected by increase in the level of the most common oxidative modification of ribonucleotides, 8-hydroxyguanosine (8-OHG). Further research will be focused on the comparison of the oxidative response of the whole leaves and isolated chloroplasts, which constitute the main site of ROS production.

## additional info

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**Biology of chloroplasts and plant mitochondria**

**Poster 28**

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# Heterologous Synthesis of Thylakoid Lipids: An *in vivo* Platform for Studying Chloroplast Proteins

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Thylakoid membranes of chloroplasts and cyanobacteria are characterized by high abundance of the non-bilayer lipid monogalactosyldiacylglycerol (MGDG). In many cyanobacteria, its precursor monoglucosyldiacylglycerol (MGLcDG) is also present. While plants synthesize MGDG in a single step from UDP-galactose, cyanobacteria utilize a seemingly inefficient two-step pathway: MGLcDG is first synthesized from UDP-glucose and then epimerized to MGDG. The evolutionary conservation of this additional step suggests an important role for the galactose headgroup in MGDG over its glucose precursor. However, the reason for galactose being the main component of thylakoid lipids remains unresolved. Studies on MGDG-lacking mutants are challenging due to its structural role, resulting in lethality or severe pleiotropic effects. To overcome this, we developed a system for heterologous synthesis of MGDG and MGLcDG in *Escherichia coli* expression strains. This provides a platform for *in vivo* studies on comparing the roles of MGDG and MGLcDG in folding and function of chloroplast proteins in a simplified model environment.

## *additional info*

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**Biology of chloroplasts and plant mitochondria**

**Poster 29**

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# The influence of strong irradiation on the photosynthetic activity of lichen photobionts

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We studied the effect of intense irradiation on photobiont photosynthesis in two epigeic lichens from open habitats but distinct photoprotective traits: melanized *Cetraria aculeata* and usnic *Cladonia uncialis*. We applied a one-hour exposure to sunlight, VIS, and UVA to assess the effect of high light stress on photobiont activity. Radiation was applied separately using artificial sources, and in combination under natural sunlight at the lichen site. We monitored chlorophyll fluorescence (OJIP, NPQ), 77K spectra, and pigment content during recovery. *Cetraria aculeata* reached maximum PSII efficiency (FV/FM) at higher light intensity than *C. uncialis* (1500 vs 1000  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Separate exposure to VIS (1800  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) and UVA (18  $\text{W m}^{-2}$ ) reduced photosynthetic parameters in JIP test, and altered the shape of fluorescence induction curves. The effects were evident just after the irradiation. Full recovery of fluorescence parameters was observed only after 18 hours in case of both species. UVA had a milder impact than VIS, and natural sunlight. UVA increased pigment content in *C. aculeata* by ~20%. Such studies are essential for understanding the response of lichens to climate change, where intense radiation and abrupt weather events may limit their development in previously stable habitats.

**Biology of chloroplasts and plant mitochondria**

**Poster 30**

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# The role of selected metabolites related to carbon metabolism in the response to dehydration and rehydration in a glycophyte *Arabidopsis thaliana* and a halophyte *Thellungiella halophila*

The plant tolerance to drought stress and rehydration in *Arabidopsis thaliana* and *Thellungiella halophila* is influenced by the level of accumulated TCs, and corresponds with changes in the levels of other metabolites of the shikimate-phenylpropanoid pathway and metabolites with antioxidative and osmoprotective properties, which may functionally cooperate with TCs. This study aimed to investigate how photochemical processes and selected primary metabolites can influence tissue dehydration and the rehydration ability. This study examined the leaves of: a glycophyte *A. thaliana* with different TC compositions (wild-type and *vte1* mutant with significantly limited TC synthesis) and a halophyte *T. halophila* exposed to soil drought (~20% ppw) and after restoration of optimal hydration (~75% ppw), and optimally hydrated control plants. The results indicate a relationship between TC levels and the chloroplastic electron transport chain and energy dissipation. Differentiated levels of metabolites including amino acids, which are linked with carbon metabolism, and varying temporal dynamics of their accumulation, as well as TC-related metabolic loops affect tissue dehydration tolerance and rehydration capacity. *T. halophila* exhibits higher baseline levels of selected primary metabolites and a broader spectrum of metabolic changes. The results also suggest that TC-related changes in carbon metabolism that are associated with maintaining redox balance.

**Biology of chloroplasts and plant mitochondria**

**Poster 31**

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# Can Polyprenols with Altered Chain Length Replace Native Polyprenols in Chloroplasts during the Heat Stress Response?

Cis-prenyltransferases (CPTs) catalyze the sequential addition of isoprene units (i.u.) to form polyisoprenoid chains of defined lengths. In *Arabidopsis thaliana*, CPT1 and CPT6 are root-expressed ER enzymes producing long-chain (18–23 i.u.) and a single short-chain (7 i.u.) polyisoprenoids, while chloroplast-localized CPT7 synthesizes medium-chain (9–11 i.u.) polyprenols in leaves. This study investigated whether subcellular localization of CPTs influences length of polyisoprenoid products. To address this, hybrid enzymes HYB7-1 and HYB7-6 were engineered by fusing the catalytic domains of CPT1 and CPT6 with the chloroplast-targeting signal of CPT7. HPLC analysis of polyisoprenoids from *Arabidopsis* expressing hybrid CPTs revealed that subcellular relocalization had a moderate effect on product chain length: chloroplast-localized HYB7-1 produced shorter polyprenols than CPT1 but longer than those produced by CPT7, while HYB7-6 generated longer products than CPT6 but still shorter than those of CPT7. Functional complementation assays in the *cpt7* mutant background under heat stress demonstrated that polyprenols of altered chain length could not substitute for native chloroplast-derived polyprenols. These results suggest that polyisoprenoid chain length is primarily determined by enzyme catalytic activity rather than subcellular localization and that only polyprenols of specific lengths can perform defined cellular functions, with no functional compensation by differently sized homologs.

## additional info

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**Biology of chloroplasts and plant mitochondria**

**Poster 32**

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# Identification of the yellow stem gene underlying chlorophyll deficiency and dwarf growth habit in cucumber

Climate change poses an imminent threat to agriculture, driving the need for resilient crops cultivars capable of maintaining high yields under stress conditions. Cucumber (*Cucumis sativus* L.), a widely cultivated vegetable crop, is particularly susceptible to high temperatures and drought. While dwarf cucumber cultivars are suitable for mechanical harvesting and maintaining productivity, such traits remain rare. This study focuses on a chlpl cucumber line, which carries the recessive yellow stem (ys) gene, associated with complex phenotype: yellow cotyledons, stems, leaf petioles and leaf veins; shortened stem length; reduced chlorophyll content in leaves; and structural abnormalities in chloroplast thylakoids. To identify the genetic basis of this phenotype, the wild-type B10 line was crossed with the chlpl, and an F<sub>2:3</sub> mapping population was developed. The ys gene was mapped to chromosome 4 and subsequent fine-mapping identified a candidate gene encoding a putative SANT/Myb-like transcription factor that contains a domain of unknown function DUF3755. The ys allele harbors a nucleotide substitution resulting in an amino acid change at a highly conserved asparagine residue within this domain, indicating its likely importance for protein function. This work provides new insights into the genetic regulation of plant growth in cucumber and may contribute to future breeding.

## additional info

This research was financially supported by the Polish Ministry of Agriculture and Rural Development under the project "Basic research for biological progress in crop production", Task 33 "Identification of genes controlling plant growth architecture of cucumber (*Cucumis sativus* L.)".

**Advances in plant structure and development**

**Poster 33**

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# Are root hairs necessary for water uptake in barley?

Modern breeding programs must meet climate change requirements, increase yield while struggling with drought and intensive fertilization (Tsang et al., 2024). As there is a consensus that root hairs are engaged in nutrient uptake, plant stability, and interactions with symbiotic bacteria, their role in water uptake is still under discussion (Cai & Ahmed, 2022). Root hairs are cylindrical protrusions of trichoblasts. Here we present the genetic, bioinformatic, and physiological analysis of the barley mutant with sparsely located root hairs. The mutant carries a premature STOP codon in the barley HvRTH3 gene (HORVU.MOREX.r3.4HG0383400), homolog of the maize rth3 gene (Hochholdinger et al., 2008). The microscopy, co-segregation, gene ontology analyses, and gene expression profiling showed that the HvRTH3 gene regulates root hair development in barley. Although some reports indicate the role of root hairs in water acquisition, by enlarging the root surface, in our experiment hvrth3.h plants perform comparably to wild type in drought and controlled conditions. Considering this, we presume that root hairs can be dispensable for water acquisition.

## *additional info*

References: Cai, G. and Ahmed, M. A. (2022). J. Exp. Bot. 73(11), 3330-3338 Hochholdinger, F. et al., (2008). Plant J. 54(5), 888-898

This work was supported by the European Union within the Seventh Framework Programme under the project no. 289300 'EURoot: Enhancing resource Uptake from Roots under stress in cereal crops' and the National Science Center (NCN) grant: DEC-2023/07/X/NZ9/00721

## **Advances in plant structure and development**

### **Poster 34**

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# ROS-dependent proteome reprogramming during apple (*Malus domestica* Borkh.) seed dormancy release

Dormancy of apple (*Malus domestica* Borkh.) seeds is alleviated through exposure to cold stratification. To investigate the underlying mechanism, seeds were subjected to low temperatures in moistened sand for 7, 14, 21, and 40 days. As stratification was prolonged, a gradual increase in RBOHC expression in embryonic axes was observed. It was reflected by rising levels of reactive oxygen species (ROS). ROS-dependent modifications affect proteins, with carbonylation serving as a key representative. The accumulation of carbonylated proteins was particularly evident after 14 days of stratification. This modification targeted proteins that became functionally obsolete at specific physiological stages or interfered with the dormancy-breaking processes. The study also provided evidence for the carbonylation of seed biotin-containing proteins. Given the limited understanding of biotin release mechanisms in plants, this specific modification is proposed as a potential initiator of the process. At an early stage of dormancy release, an increase in proteasome activity was detected, despite relatively low ATP levels. This suggests the involvement of the 20S proteasome in regulating levels of carbonylated proteins, including storage proteins. These findings highlight the importance of ROS-mediated proteome remodelling in embryonic axes, which accompanies dormancy release of apple seeds.

## additional info

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**Advances in plant structure and development**

**Poster 35**

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# Divergent *Droseras* - Trap leaf specialization in *Drosera rotundifolia*

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The carnivorous plant *Drosera rotundifolia* has active adhesive traps with different types of stalked glands i.e. central, peripheral, and highly motile snap tentacles. We observed that tentacle type patterns in plants and populations notably differ. This study systematically examines these divergences across 22 populations from Austria, Germany, and Czech Republic, and sets them in relation to geographical, geological, and ecological parameters. Differences are highly correlated with increased geographical distance and depend on bog type, water regime, and substrate. However, tentacle patterns were best explained by different frequencies of two leaf types: prostrate basal leaves that possess abundant snap tentacles ( $9.0 \pm 5.1$  per leaf), and seemingly adapted to capturing crawling prey. This leaf type is complemented by erect leaves, exhibiting significantly less snap tentacles ( $3.1 \pm 5.0$  per leaf), more peripheral tentacles, and appear to target flying prey. This indicates that snap tentacles particularly contribute to the trapping of crawling but not of flying prey, in good accordance with the lack of snap tentacles in *D. anglica* from the same habitat with only erect leaves. Thus, each specimen of *D. rotundifolia* exhibits so far overlooked trap specializations similar to the well known pitcher plant *Nepenthes*.

**Advances in plant structure and development**

**Poster 36**

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# Temperature acclimatization mechanisms in the psychrotolerant microalga *Coccomyxa subellipsoidea* C-169

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*Coccomyxa subellipsoidea* C-169 is a unicellular green alga adapted to a challenging Antarctic environment. It is a psychrotolerant (with optimum growth temperature 20°C) characterized by a wide range of temperature tolerances (up to +30°C) and high lipid content. As the first sequenced eukaryotic microorganism from a polar environment, it can serve as an attractive model for studies of the acclimatization to different temperatures and finally adaptation mechanisms to cold. These results aim to clarify the acclimatization mechanisms that enable the psychrotolerant green alga C-169 to grow in a broad temperature spectrum. The contents of various biochemical compounds in cell, the lipid composition of the entire cells biological membranes, the thylakoid fraction, the electron transport rate and PSII efficiency were shown. The results demonstrate an acclimatization mechanism that is specific for C-169 and that allows the maintenance of appropriate membrane fluidity. It is achieved almost exclusively by changes within the unsaturated fatty acid pool. This ensures, for example, an effective transport rate through PSII. These findings add substantially to our understanding of the acclimatization of psychrotolerant organisms to a wide range of temperatures and prove that this process could be accomplished in a species-specific manner.

## additional info

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**Advances in plant structure and development**

**Poster 37**

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# Identification and characterization of barley overgrowth mutant – *mg13*

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The aim of this study is to identify and functionally characterise the HvMG13 gene in barley (*Hordeum vulgare*). The *mg13* mutation was identified based on its phenotype, as the plants carrying the mutation were significantly taller than the parental Sebastian cultivar. The *mg13* mutant was selected from the HorTILLUS population, which was developed through chemical mutagenesis. Genetic analyses revealed that the *mg13* mutation is recessive, but it is not located within the coding sequences of the two candidate genes associated with gibberellin signalling, SLENDER1 (SLN1) and SPINDLY1 (SPY1). To confirm the effect of the selected mutation on the barley phenotype and to obtain material necessary for mutation identification, a series of crosses were performed between *mg13* mutant and four barley cultivars: Avatar, Barke, Jessie, and Planet. Preliminary observations revealed that the *mg13* mutation leads to increased plant height and delayed plant development. Moreover, it was shown that decreased chlorophyll content in mutant leaves is not related to the *mg13* mutation, as a similar reduction in chlorophyll content was observed in heterozygous plants from F1 generation. The hydroponic culture allowed for the investigation of root system architecture in mutant plants and revealed a reduction in total root length compared to Sebastian.

**Advances in plant structure and development**

**Poster 38**

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# The Spliceosome at the Heart of Seed Life and Death: Roles of AtCWC22 and CYP95 in Arabidopsis

Proper seed development in *Arabidopsis thaliana* relies on tightly coordinated transcriptomic, metabolic, and epigenetic programs. Among the key regulators are spliceosomal proteins involved in pre-mRNA splicing, including AtCWC22 (the Arabidopsis ortholog of human CWC22) and its interactors AtCWC21 and cyclophilin CYP95. Here, we identify AtCWC22 as essential for embryogenesis: homozygous *cwc22* mutants are embryo-lethal, with heterozygotes producing defective seeds that arrest at the late globular stage. By contrast, *cwc21* and *cyp95* loss-of-function mutants exhibit normal embryogenesis and germination, indicating distinct roles. Single-seed RNA-seq during embryogenesis revealed AtCWC22-dependent gene networks, suggesting broader functions beyond splicing. Notably, *cyp95* mutant seeds misregulate photosynthesis- and stress-responsive genes between 5–6 days after pollination, coinciding with reduced seed longevity. Although *cwc21* and *cyp95* seeds display highly similar transcriptomes, only CYP95 appears to influence maturation through plastid-associated pathways. Overall, our findings demonstrate that spliceosomal components, particularly AtCWC22, play crucial roles not only in splicing but also in transcriptional regulation during seed development. Disruption of these components: AtCWC22 and CYP95 – compromises respectively embryogenesis, dormancy, and longevity, underscoring their potential as targets for improving seed traits in biotechnological applications.

## additional info

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**Advances in plant structure and development**

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# SnRK2.4 and SnRK2.10 redundantly control developmental leaf senescence by sustaining ABA production and signaling

Plants constantly and precisely control their growth by inducing distinct developmental programs to survive and produce high-quality offspring. Kinases of the Sucrose non-fermenting-1-Related protein Kinases type 2 (SnRK2s) family primarily take part in the response and adaptation to environmental stress factors. Notably, here we show that two ABA-non-activated SnRK2s, SnRK2.4 and SnRK2.10, are also activated in non-stress conditions in developmentally senescing leaves of *Arabidopsis thaliana*. Phenotypic, biochemical, and molecular analyses performed on single *snrk2.4* or *snrk2.10*, and double *snrk2.4/2.10* kinase mutants showed that SnRK2.4 and SnRK2.10, acting redundantly, promote developmental leaf senescence. Further, both kinases enhance ABA accumulation in senescing leaves by inducing NCED2, one of key ABA biosynthesis-related genes and modulate the expression of multiple ABA-responsive, osmotic stress, and senescence-related genes, such as the senescence master regulators ORE1, ORS1, WRKY33, WRKY75, and ANAC087. Furthermore, we show that SnRK2.4 and SnRK2.10 act upstream of MAPK signaling by enhancing the expression and activity of MAPKKK18, a leaf senescence-inducing kinase. These results document a new regulatory function of SnRK2.4 and SnRK2.10: they are activated in *Arabidopsis* leaves in response to endogenous signals and redundantly induce developmental leaf senescence by stimulating ABA production and sustaining major ABA-dependent and -independent signaling pathways.

## additional info

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## Advances in plant structure and development

### Poster 40

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# Viable microspores, seeds and hypocotyl-based plant regeneration from genetic female *Cannabis sativa* L. following STS-induced staminate flower formation

*Cannabis sativa* L. is cultivated for food, fiber, and medicine. It is also an emerging model in plant science. This species is usually dioecious, but when it comes to the production of cannabinoids and other phytochemicals highly synthesized in glandular trichomes, female genotypes are preferred. By applying chemicals, e.g. silver thiosulphate (STS), it is possible to induce staminate flower development on female plants, and ensure feminization of the population. In this study we assessed the suitability of STS-induced immature pollen grains (microspores) derived from genetic female cannabis plants for microspore embryogenesis (ME), and the potential of hypocotyls derived from feminized seeds for *in vitro* plant regeneration. We have found that microspore development is synchronized across all flower buds. We have also identified flower bud size corresponding to the uninucleate stage, optimal for ME induction. The experimental approach enabled us to generate pollen grains from female plants, which subsequently produced feminized seeds. Derived hypocotyls regenerated directly into plants. The regeneration effectiveness depended largely on hormonal composition of the medium. The regenerated plants were confirmed to be genetic females. The STS-induced microspores can be used as the basis for molecular breeding and genetic engineering of *Cannabis sativa* L.

## additional info

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## Advances in plant structure and development

### Poster 41

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# Deciphering AGPs role in Arabidopsis wood

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Arabinogalactan proteins (AGPs) are highly glycosylated ubiquitous plant proteoglycans located predominantly on the plasma membrane. They have been found to participate in a myriad of developmental and physiological processes, including plant response to abiotic stress. However, up until now, AGPs' role in wood development and functioning has been rather ambiguous. Here, we present the spatial distribution of these proteins in Arabidopsis hypocotyl wood tissue in wild type plants and transgenic lines characterized by higher and lower callose content. In order to investigate if there is any relationship between callose content (which may impacts the cellular communication) and AGPs we implemented several approaches, including immunolabeling with specific antibodies and microscopic analyses (fluorescent microscopy, confocal microscopy, Lattice Lightsheet and SIM superresolution). Our analyses are conducted in both control and drought conditions, as limited water supply can influence the AGPs' epitope distribution. Altogether, our findings implicate that AGPs may potentially influence the wood formation and functioning in Arabidopsis, what is further modulated by abiotic stress conditions.

## *additional info*

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**Advances in plant structure and development**

**Poster 42**

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# Hydrogen peroxide-mediated release of zinc from type 4 *Sorghum bicolor* metallothionein

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Metallothioneins (MTs) are ubiquitous, cysteine-rich proteins that bind heavy metal ions. Plant metallothioneins are divided into four types. Type 4 MTs (pMT4) are seed-specific proteins involved in the accumulation of zinc during seed maturation, and donate it to nascent proteins during germination. Hydrogen peroxide ( $H_2O_2$ ), present throughout seed development and increasing at germination onset, is known to oxidise thiolates and may trigger zinc release from MT4 proteins. To test this, we studied zinc mobilisation from type 4 sorghum metallothionein (SbMT4) in response to  $H_2O_2$ . SbMT4 was recombinantly expressed in *E. coli*, purified via liquid chromatography, and the metal release from SbMT4-zinc complexes was monitored by mass spectrometry. MTs can also bind cadmium - toxic zinc mimic; therefore, we also examined SbMT4-cadmium complexes to compare.  $H_2O_2$  triggered the release of both zinc and cadmium from SbMT4, but zinc was released significantly faster. These results suggest that increased  $H_2O_2$  levels during germination may promote zinc release from SbMT4, facilitating its transfer to emerging proteins. This highlights a potential role for  $H_2O_2$  in regulating intracellular zinc availability during seed maturation and germination.

## *additional info*

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**Advances in plant structure and development**

**Poster 43**

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# Quantification of hygroscopic movements of white fir (*Abies concolor*) cone scale

Among the cones of the Pinaceae family, those of *Abies concolor* (white fir) perform one of the most profound movements. All Pinaceae cones are designed to open and close, involving their hygroscopic behavior. However, the white fir cone explodes at the same time to release both the seeds and scales. Unlike in pine cone, the movements of large ovuliferous scales of white fir are extensive but their mechanism is not fully understood. This work focuses on the biomechanical behavior of the white fir scales. The 3D Digital Image Correlation (3D-DIC) was used to quantify deformation of the abaxial and adaxial surfaces of the ovuliferous scale during the transition from wet to dry. This analysis showed that during the 45 min, in the course of which the complete deformation process occurred, unevenly distributed strong longitudinal strains occurred on the abaxial side. On the adaxial surface, longitudinal strains were weaker while transverse strains dominated. Using  $\mu$ -CT, three layers were distinguished within the scale that contribute differently to this differential deformation and differ in stiffness as shown by extensometer measurements.

## additional info

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**Advances in plant structure and development**

**Poster 44**

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# Autophagy Deficiency Alters Lipid Droplet Dynamics and TAG Mobilization in Germinating Arabidopsis Seeds

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Plant lipid droplets (LDs) are dynamic storage organelles that can be degraded by the autophagy machinery to release triacylglycerols (TAGs), in a process known as lipophagy. In this study, we investigated the role of autophagy in LD mobilization during *Arabidopsis thaliana* seed germination. To assess this, we analyzed the fatty acid composition of total lipids from mature and germinating seeds of AUTOPHAGY-RELATED GENE 5 - ATG5 and ATG2 mutants, both defective in autophagosome formation and lacking the phosphorylated form of the ATG8, a core autophagy protein. Notably, the content of 20:1 fatty acid, a marker of TAG degradation, was significantly lower in atg5 and atg2 at 48, 72, and 96 hours of germination compared to wild-type plants. OLEOSIN1, the major structural protein of LDs, was also degraded more rapidly in the mutants, becoming undetectable after 24 hours, while remaining present in wild-type. Furthermore, both mutants displayed smaller and less abundant LDs in cotyledon cells during later stages of germination. These altered lipid profiles and LD behavior indicate prominent role of autophagy in LDs degradation. Our findings provide new insights into lipophagy in plant development and establish a basis for further investigation into the molecular relationship between autophagy and lipid metabolism.

## *additional info*

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**Advances in plant structure and development**

**Poster 45**

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# Cell cycle parameters as markers of *in vitro* potato plant regeneration capacity

The application of new genomic techniques or transformation using bacterial vectors requires plant regeneration in *in vitro* culture. There is a problem of poor and/or prolonged plant regeneration from shoots. Plant growth and development are based on three processes of cell cycle progression: mitosis, endoreplication and meiosis. Cell multiplication through mitosis is crucial in the process of organ growth. The cell cycle consists of four phases (G0/G1, S, G2, M), that are regulated by cyclins (CYC) and cyclin-dependent kinases (CDKs). We studied the regenerative capacity of 20 potato genotypes: 18 diploid interspecific potato hybrids and 2 potato cultivars, maintained in *in vitro* cultures. Cell cycle progression was analyzed at 9, 12, 15 and 28 days after explant transplantation. The frequency of cells in G1, S, G2 phases, the endoreduplication ratio (SCV) and the frequency ratio of nuclei after endoreduplication to 2C nuclei ( $\Sigma > 2C/2C$ ) were studied. Plant phenotypic traits such as the number, length of roots and shoots; number and size of leaves, and percentage of regenerated plants were analyzed. Significant differences in cell cycle progression, phenotypic parameters, and the expression of genes related to cell cycle progression were observed in the group of plants with high and low regeneration capacity.

## additional info

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**Advances in plant structure and development**

**Poster 46**

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# Methylation of IAA in a light-dependent regulation of Arabidopsis seedling development

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Methylation of indole-3-acetic acid (IAA), a major plant auxin, is a reversible modification that may modulate auxin level and activity. The enzyme IAMT1 (IAA CARBOXYL METHYLTRANSFERASE 1) converts IAA into its methylated form (meIAA), while MES17 (METHYLESTERASE 17) hydrolyzes meIAA back to free IAA. Although meIAA was long been viewed as an inactive, storage form of auxin, recent studies suggest it may have additional, unexplained developmental functions. We investigated how altering meIAA metabolism affects early seedling development in *Arabidopsis thaliana* grown under different light conditions and hormone treatments. We analyzed mutants deficient in IAMT1 or MES17 and lines overexpressing IAMT1. Seedlings were grown under long/short day or darkness, with or without IAA or meIAA. To study spatial distribution of meIAA metabolism, we used an IAMT1::GUS reporter line and performed immunolocalization of MES17. We found genotype- and light-dependent differences in root and hypocotyl growth inhibition, as well as in gravitropic and phototropic responses. Additionally, we observed that IAMT1::GUS expression and tissue distribution of MES17 were modulated by both light and hormone treatment. These results suggest that auxin methylation and demethylation pathways dynamically respond to environmental cues, modulating auxin-dependent seedling development.

*additional info*

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**Advances in plant structure and development**

**Poster 47**

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# Implementation of lattice Lightsheet microscopy (LL7) for fast and gentle scanning of varied plant specimens at a high resolution

One of the crucial problems associated with plant visualisation using a wide-field fluorescent and confocal microscopes are phototoxicity and photobleaching. To overcome these problems a classic lightsheet microscopy for deep, fast and gentle scanning was invented. However, it is characterised by low resolution imaging. Therefore, with the aim of expanding the resolution capabilities Lattice Lightsheet 7 (LL7) microscopy was lately introduced. This innovative system, the first so far in Poland, was installed in the Lattice Lightsheet Microscopy Lab at the Faculty of Biological Sciences, University of Wrocław. It enables fast and high-resolution imaging at a wide range, with low phototoxicity and photobleaching. Notably, each scanning results in hundreds and thousands of images rapidly generated that can be almost immediately converted into 3D projections. Moreover, data quantification and detailed analyses can be easily performed with the use of Zen software. Up to now we have generated images of both living and fixed plant samples, including roots, leaves and varied hand-cut, vibratome and paraffin-embedded anatomical sections. The goal of these visualisations was e.g. to localize cytoplasmic and plasma membrane proteins and to track molecule movement within and between the cells.

## *additional info*

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# Identification of a scp gene controlling super compact plant architecture in cucumber

Plant architecture is important in plant breeding, influencing crop growth, yield, and stress tolerance. The altered plant architecture affects light perception, carbon assimilation, and dry matter accumulation, which can ultimately result in increased yields per cultivation area. It directly determines the workload in crop management and harvesting. Here, we report the research progress on cucumber line L505, characterized by super compact phenotype controlled by a single recessive gene scp, previously described by Niemirowicz-Szczyt et al. (1996). A segregating F2:3 population was developed from a cross between the L500 wild-type line and the L505 line. A genomic region associated with super compact phenotype was identified on chr4 using phenotyping and high-throughput genotyping. Fine-mapping further narrowed this region, leading to the identification a candidate gene for scp. Subsequent analysis revealed that the candidate gene encodes a protein with P450 cytochrome domain, which is involved in the brassinosteroids biosynthesis pathway. RT-qPCR expression profiling revealed differential regulation of this gene, with upregulation in petioles and flowers and downregulation in roots, leaves, and main stem. These findings provide novel insights into the molecular basis of the super compact growth habit of cucumber and identify potential targets for the genetic improvement of plant architecture in cucurbits.

## *additional info*

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**Advances in plant structure and development**

**Poster 49**

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# Nature versus Nurture in Carnivorous Plants (Field transplants reveal carnivory traits flexibility in *Drosera rotundifolia*)

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*Drosera rotundifolia* is a carnivorous plant growing in nutrient-poor habitats across the Holarctic, adapting to varied ecological niches. Tentacles responsible for prey capture show pronounced differences between habitats. Whether tentacle patterns are genetically or environmentally determined remains unclear. We conducted a transplantation experiment transposing plants from a wet sandpit (SP) in the Czech Republic to two ombrogenic peat bogs (PB) in Austria. Measurements included growth parameters, tentacle patterns, and prey capture. Originally, leaf size and total tentacles were higher at SP with less abundant snap tentacles. After transplantation from SP to PB, leaf size, total tentacles and prey capture decreased significantly, while snap tentacles increased. After transplantation from PB to SP, an increase in leaf size and prey capture, more total, but less snap tentacles were observed. Environmental conditions are harsher at PB compared to SP, with lower temperature and fewer nutrients. Overall growth and prey capture of plants from SP were inhibited. Snap tentacles were more frequent early in the growth season, supporting this trend. In contrast, plants from PB cannot fully utilize the favorable conditions at SP. Thus, strong genetic determination is probable, supported by a correlation between morphological and geographic distance, found in a parallel study.

**Advances in plant structure and development**

**Poster 50**

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# Artificial ageing of apple (*Malus domestica* Borkh.) seeds corresponds with alterations in nitrate reductase activity in embryonic axes

Seeds serve as crucial dispersal units, and their viability significantly influences the growth and development of mature plants. Seed ageing, integral to plant ontogenesis, affects various morphological, physiological, and biochemical aspects and is linked to disturbances in nitric oxide (NO) metabolism. Despite their low metabolic activity, dormant apple seeds (*Malus domestica* Borkh.) demonstrate signs of ageing when exposed to elevated temperatures in moistened sand for over 14 days. This work aimed to investigate changes in nitrate reductase transcript and protein levels, as well as activity in embryonic axes of apple seeds subjected to accelerated ageing, along with evaluating nitrate and nitrite (non-enzymatic NO source) concentrations. Apple seeds were artificially aged for 7, 14, and 21 days at 35°C, followed by embryonic axes isolation. Non-aged, dormant seeds were used as the control. Embryos isolated from aged seeds germinated slowly and exhibited multiple morphological abnormalities in developing seedlings. In the embryonic axes of aged seeds, nitrate and nitrite concentration fluctuations were noted. These changes go along with the abundance and activity of nitrate reductase. It was observed that the relative transcript level of nitrate reductase increased only after 14 days of ageing.

## additional info

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**Advances in plant structure and development**

**Poster 51**

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# Comparative studies of calreticulin3a/b gene expression during pollen development in *Petunia*

The plant calreticulin (CRT) protein family consists of evolutionarily conserved, calcium-binding chaperones located primarily in the endoplasmic reticulum (ER). They are involved in protein folding and quality control on the ER, regulation of calcium homeostasis, and plant responses to biotic and abiotic stresses. In contrast to animals, plant cells express three CRT isoforms exhibiting functional specialization: CRT1, CRT2, and the plant-specific CRT3. We recently cloned and characterized two novel genes from the model plant *Petunia hybrida*: PhCRT3a and PhCRT3b. We also showed that siRNA-mediated post-transcriptional silencing of PhCRT3a in pollen tubes causes significant morphological and ultrastructural defects, resulting in impaired pollen tube growth *in vitro*. Based on the results of our long-term studies on the role of CRT proteins in the generative reproduction of angiosperms, we assumed that both identified genes, PhCRT3a and PhCRT3b, are expressed during pollen formation in the anther. Therefore, we decided to investigate the expression profiles of these genes during successive stages of microsporo/gametogenesis in *Petunia* anther. For this purpose, we used semi-quantitative PCR (sqPCR) and fluorescence in situ hybridization (FISH) to analyze the expression patterns of PhCRT3 and PhCRT3b in the context of their potential role in male gametophyte development.

## *additional info*

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**Poster 52**

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# Alternative splicing in auxin-dependent processes: Time to change auxin synthesis

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Alternative splicing (AS), an essential post-transcriptional mechanism, enables the generation of multiple mRNA isoforms from a single pre-mRNA. While prevalent in both plants and animals, the functional significance of most AS events remains largely unresolved. Many alternative mRNAs may not be translated; however, when they are, AS can alter a protein's interactome and thereby modulate its function. Based on our previous work on the AS of the auxin transporter PIN7, we established a set of criteria (evolutionary conservation, translation potential, and phenotypic relevance) to identify AS events with high functional potential. We selected ASA1 and ASB1, which encode the  $\alpha$ - and  $\beta$ -subunits of the ANTHRANILATE SYNTHASE complex, the enzyme catalyzing the rate-limiting step in the tryptophan-dependent indole-3-acetic acid (IAA) biosynthesis. Here we investigate how AS modulates auxin biosynthesis through isoform-specific regulation of ASA1 and ASB1. Using fluorescent reporters, we observed distinct localization patterns of splice variants in planta, suggesting functional divergence. Genetic complementation assays in null backgrounds (*asa1-1*, *asb1-1*) is used to assess the ability of individual and combinatorial isoforms to rescue auxin-related phenotypes. Moreover, structural modeling suggests isoform-specific effects on subunit interaction and active site conformation within the ASA1/ASB1 tetramer. These predictions are being validated through protein interaction assays.

## *additional info*

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**Advances in plant structure and development**

**Poster 53**

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# PCD as a possible reason for poor seed germination in selected species of the *Viola* genus

Seed germination is a complex process and one of its stages involves nutrient release from the endosperm, which requires programmed cell death (PCD). Many species of the genus *Viola* show low seed germination frequency (e.g. *V. odorata*, ~ 0%) though there are also species with a relatively high germination frequency (e.g. *V. x wittrockiana*, 56.5%; *V. tricolor*, 27.5%, after cold stratification). To determine whether the PCD is connected with seed germination, seeds of violets with high and low germination capacity were analyzed. The localization and intensity of PCD in seeds were determined by the TUNEL assay and Western Blot analysis. The results showed that PCD occurred in endosperm and embryos of violets with low and high germination capacity. In seeds of *V. odorata*, PCD was present rather in parts of the endosperm near the seed coat, whereas in seeds of *V. x wittrockiana* and *V. tricolor*, also in the endosperm surrounding the embryo. The activation of PCD in the embryos might be related to the xylogenesis and the presence of short-lived cotyledons. These results indicate that PCD may contribute to triggering seed germination. Further experiments including germinating and non-germinating seeds with broken relative dormancy will verify this hypothesis.

## additional info

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## Advances in plant structure and development

### Poster 54

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# Characterization of neutral ceramidase activity and membrane microdomains in *Brachypodium distachyon*

Sphingolipids are crucial structural and signaling components in plants, playing key roles in membrane organization, stress response, and programmed cell death. Ceramidases regulate sphingolipid homeostasis by hydrolyzing ceramides into long-chain bases (LCBs) and fatty acids. While animal ceramidases are classified as acidic, neutral, or alkaline, only neutral and alkaline homologs have been identified in plants. In *Arabidopsis thaliana*, three neutral ceramidases (NCERs) have been functionally characterized, and their loss of function leads to lipid imbalance and triggering programmed cell death. In this study, we examined sphingolipid-related processes in the monocot model *Brachypodium distachyon*, focusing on membrane lipid microdomains and neutral ceramidase activity. Lipid rafts are sphingolipid-rich microdomains that mediate protein sorting and signaling. Using the polarity-sensitive dye di-4-ANEPPDHQ, we successfully visualized lipid rafts as ordered microdomains in living root cells, without affecting their viability. Finally, we identified and characterized the putative NCER homolog from *B. distachyon* (BdNCER). Its sequence analysis showed the presence of a conserved amidase domain. We also confirmed the activity of BdNCER in ceramide hydrolysis by its heterologous expression in yeast. Overall, our findings provide the first experimental evidence on membrane microdomain organization and ceramidase function in *B. distachyon*, advancing our understanding of sphingolipid regulation in monocots.

## additional info

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**Advances in plant structure and development**

**Poster 55**

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# Impact of LOX inhibition and wounding on alkaloid biosynthesis in *Papaver somniferum* L.

The mechanisms underlying systemic signalling in plants following injury or pathogen attack, which leads to altered secondary metabolite production in target tissues, remain poorly understood. Lipoxygenase (LOX) and the octadecanoid pathway, where LOX plays a central role, are potential candidates for mediating these responses. Despite the pathway's relevance to secondary metabolite synthesis via signal transduction, LOX's influence on plant alkaloid accumulation has not been thoroughly explored. This study investigates LOX's role in modulating benzyloisoquinoline alkaloid (BIA) biosynthesis in 6-week-old opium poppy plants (*Papaver somniferum* L.) at both transcriptional (by real-time qPCR) and metabolite levels (using HPLC-MS) following mechanical injury. LOX inhibition significantly enhanced the accumulation of reticuline, salutaridine, and codeine. Elevated reticuline levels suggest activation of not only the morphinan branch but also other BIA biosynthesis pathways. Wounding combined with LOX inhibition triggered markedly higher codeine production compared to intact plants. These findings highlight LOX as a key modulator of BIA metabolism, linking wound-induced signalling to the biosynthesis of alkaloids. The results provide novel insights into plant defence mechanisms and potential strategies for optimising secondary metabolite production in medicinal plants.

## additional info

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A part of the experiments in this work was carried out in the Toxicological and Antidoping Center at the Faculty of Pharmacy, Comenius University, Bratislava.

## Plants and abiotic stresses

### Poster 56

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# Methyl jasmonate induced rosmarinic acid production in *Perilla frutescens* *in vitro* cultures

Rosmarinic acid (RA) is a bioactive phenolic compound with antioxidant, antimicrobial, anti-inflammatory, and potential anti-cancer properties, found in *Perilla frutescens* L. (Britt.) of the family Lamiaceae. Methyl jasmonate (MeJA), a plant-signalling molecule derived from jasmonic acid, is a key mediator in stress responses. It is used as a promising strategy in biotechnology to enhance secondary metabolites production in plants, including RA, through elicitation. Prepared *in vitro* suspension cultures of *Perilla frutescens* were treated with MeJA at three concentrations (50, 100, and 150  $\mu$ M) in a time-dependent manner. The effect of MeJA on RA production was assessed through metabolite quantification using HPLC-DAD and transcriptional analysis of key enzymes in the tyrosine-derived pathway of RA biosynthesis. Our results indicate that MeJA treatment significantly increased RA content in *Perilla frutescens* suspension cultures after 72 hours of MeJA treatment at 100  $\mu$ M and 150  $\mu$ M concentrations, while shorter exposures and lower concentrations had minimal effect. Gene expression of biosynthetic enzymes correlates with RA content. These findings highlight the importance of optimizing both elicitor concentration and exposure time to maximize secondary metabolite yields in plant cell cultures, offering a promising approach for sustainable production of bioactive compounds.

## additional info

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## Plants and abiotic stresses

### Poster 57

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# How Tobacco Roots Respond to Zinc Deficiency: tissue-specific expression of NtZIP1-like, NtZIP4B and NtZIP5B genes

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One of the key nutritional challenges in the modern world is the inadequate intake of essential micronutrients, particularly those derived from plant-based sources. Our goal is deciphering how dicotyledonous plants redistribute zinc (Zn) under deficiency, addressing gaps in understanding its internal distribution. A hydroponic system was used to study Zn redistribution in tobacco. After 2.5 weeks of growth with 1  $\mu$ M Zn (point 0), some plants were transferred to Zn-deficient conditions. Six days later, both groups showed similar phenotypes despite a fourfold biomass increase, indicating efficient redistribution of Zn accumulated prior to deficiency. These findings suggest that Zn accumulated by the plants up to time point 0 is effectively redistributed to developing tissues. ZIP (ZRT-IRT-like) proteins are transmembrane transporters involved in Zn homeostasis. Their expression is often upregulated under Zn deficiency, highlighting the need to examine their spatial expression to clarify roles in Zn redistribution. For this purpose, we utilized transgenic tobacco lines expressing the GUS reporter gene under the control of the NtZIP1-like, NtZIP4B, or NtZIP5B promoters. These transgenic lines were cultivated following the experimental scheme described above. At the conference we will present site-specific patterns of pZIP1L, pZIP4B and pZIP5B promoter activity in whole roots

## *additional info*

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**Plants and abiotic stresses**

**Poster 58**

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# Timing, not magnitude defines transcriptomic resilience

Chilling stress limits the early growth of C<sub>4</sub> bioenergy grasses in temperate climates. To understand how temporal dynamics shape chilling resilience, we analysed transcriptomic responses in two contrasting *Miscanthus sinensis* genotypes: Ms12 (low chilling tolerance) and Ms16 (high chilling tolerance). Using a stepwise chilling-recovery regime, we traced changes in the expression of genes linked to cell wall metabolism. Ms12 genotype mounted a rapid and transient transcriptional burst at early stages of chilling exposure characterized by extensive induction of cellulose synthase and glycosyltransferase genes. In contrast, Ms16 genotype showed delayed but sustained activation of key factors, particularly those involved in energy metabolism, secondary cell wall reinforcement and hormonal coordination. For HCT genotype the expression of several genes (2930 transcripts) was observed in the later stages of stress exposition (day 36) while for LCT genotype the observed response was fragmented and short-lived. Our findings demonstrate that chilling resilience in *Miscanthus sinensis* depends not on early response magnitude, but on the integration and temporal coordination of stress mitigation and recovery pathways. This study offers a temporal blueprint for chilling resilience in perennial grasses, paving the way for climate-smart breeding strategies in biomass crops.

## additional info

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## Plants and abiotic stresses

### Poster 59

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# Toxic effect of cadmium or zinc on photosynthesis in selected *Biscutella laevigata* populations

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The present study aimed to investigate how Cd or Zn affects the photosynthesis and pigment content of the leaves of selected populations of *Biscutella laevigata*. The Hoagland medium was used in the experiment for hydroponic cultures of *B. laevigata* from two metal-contaminated sites (Dołki and Bukowno) and a reference site (Kozubow). The plants, after growing under controlled conditions for 2 months, were treated with 5  $\mu\text{M}$  Cd or 50  $\mu\text{M}$  Zn for 2 weeks, and then the activity of the photosystem II and pigment content were measured. The results showed that the treatment of plants with Cd or Zn negatively affected photosynthetic parameters and chlorophyll content of all tested populations. However, plants from contaminated sites (Dołki and Bukowno) showed a better physiological status under control conditions than plants from Kozubow. Both tested metals similarly damaged photosystem II in plants from Kozubow. Populations from Dołki and Bukowno showed similar resistance to 50  $\mu\text{M}$  Zn in the medium, while Cd showed a higher toxic effect on photosystem II in plants from Dołki than Bukowno. The results showed different Cd and Zn hypertolerance strategies in selected populations of *B. laevigata*, while suggesting the need for further experiments on the mechanisms studied.

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**Poster 60**

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# Phospholipid signaling of tobacco BY-2 cell suspension lines adapted to salt/osmotic stress

Osmotic and salt stress activate several phospholipid signalling pathways in plant cells. We focused on tobacco BY-2 cells treated with stress agents and compared these with BY-2 cells adapted and maintained under severe stress conditions for 15 years. We have studied the effects of salt- and mannitol stress adaptation on phospholipid signalling, lipidome and plasma membrane properties. Results from mass spectrometry showed that BY-2 cells underwent substantial changes in their lipid composition upon adaptation to stress. Labeling with molecular rotor mechanoprobe N<sup>+</sup>-BDP indicated stronger mechanical restriction for rotations in the salt-adapted BY-2 cells, likely due to tightly packed membrane lipids or compression of the plasma membrane due to hyperosmotic stress. *In vivo* 32P-orthophosphate (32Pi)-labelling of BY-2 cells and TLC- and Phosphoimaging analyses were used to compare phospholipid content of the various cell suspensions and to monitor their responses to salt- and osmotic stress. The results revealed differential responses for PA and PI(4,5)P<sub>2</sub>, while other signaling phospholipids and structural phospholipids remained on a similar level. Our results indicate that adaptation to either salt- or osmotic stress modifies the stress perception and activation of downstream pathways, which are likely involved in the protection mechanism of adapted cells to rapid environmental changes.

## additional info

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### Poster 61

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# Redox Status as a Metabolic Switch Regulating Senescence *via* Polyamine Catabolism During Premature Senescence in Barley Leaves

Senescence enables plants to respond to stress, regulating the survival or programmed cell death. Polyamines are key regulators of this process. Polyamine catabolism increase hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) levels, shifting the redox state toward more oxidative, and promote senescence. Maintaining optimal polyamine levels and inhibiting their catabolism can delay senescence. In this study, we examined the cellular redox status as a switch controlling senescence via the polyamine catabolism. Barley plants were subjected to Dark-Induced Leaf Senescence (DILS), characterized by a shift toward an oxidative redox state. Low oxygen conditions (LOC, 1% O<sub>2</sub>) promoted reducing environment in treated plants. Leaves from 7-day-old seedlings were analysed after 0 (control), 3, 7 and 10 days of dark incubation. To monitor senescence, maximum quantum yield of PSII, a good senescence marker, was measured using Photon Systems Instruments. Redox status was assessed via H<sub>2</sub>O<sub>2</sub> content and antioxidant capacity (Trolox Equivalent Antioxidant Capacity assay). Polyamines and their catabolic product - 1,3-diaminopropane - were quantified using gas chromatography-mass spectrometry. During DILS, polyamine oxidation was accompanied by disturbance in redox homeostasis, contributing to senescence. In contrast, LOC slowed senescence progression by preserving polyamine levels and delaying their catabolism.

## *additional info*

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## Plants and abiotic stresses

### Poster 62

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# Uncovering the key genes in barley's response to aluminum stress

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Aluminum (Al) is the third most abundant element in the Earth's crust, following oxygen and silicon. In neutral or alkaline soils, Al is incorporated into various non-toxic minerals. However, aluminum dissolves under acidic conditions, forming highly reactive and phytotoxic species known as  $\text{Al}^{3+}$  ions. Plants have evolved various response strategies to mitigate Al toxicity, including the exclusion of organic acid anions (OAs) into the rhizosphere to chelate  $\text{Al}^{3+}$  and the sequestration of Al into the vacuole for detoxification. The Al stress response is primarily regulated by the transcription factor STOP1, which controls the expression of genes involved in Al tolerance mechanisms. Additionally, the ABC-family transporter ALS1 plays a crucial role in Al detoxification by mediating the transport of  $\text{Al}^{3+}$ :OA complexes into the vacuole. Our study focuses on the Al stress response in barley, which is known to be one of the most sensitive crop species to Al toxicity. Here, we present preliminary findings on the role of barley STOP1, ALS1.1, and ALS1.2 genes in the Al stress response. Our findings contribute to a better understanding of the function of these genes in barley's response to Al stress, which, to our knowledge, has not been previously investigated in this species.

## *additional info*

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## Plants and abiotic stresses

### Poster 63

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# Long-term effects of cadmium and nickel on key metabolic enzymes of agricultural plants of the orders Fabales and Poales

In Hungary, the most eroded soils are located in the Hajdúság, where an increase in Ni concentration has been recorded in recent decades. For providing of crop yield, the use of NPK fertilizers is unavoidable, which have a significant Cd content due to the production technology. The long term effects of cadmium on our economic crops are -absolutely- negative, with a reduction in biomass production, early chlorosis and, in more severe cases, necrosis. Nickel is essential for plants at low levels, but at higher concentrations it has a toxic effect, leading to chlorosis. Our experiments investigated the long-term effect of Ni and Cd gradient (0-50 ppm) on 21-day-old sand-cultured pea and barley and 14-day-old YFP-peroxi Arabidopsis (used as a model plant). In addition to measuring length and wet weight, protein content, ferric reducing antioxidant power, acidic and alkaline phosphatases, triose phosphatase,  $\beta$ -NAD diphosphatase, polyphenol oxidases and peroxidases activity were determined. In both species acidic phosphatase,  $\beta$ -NAD diphosphatase and polyphenol oxidases activities decreased after Cd treatment. In pea alkaline phosphatase activities were increased by both metals, while decreased in barley. Treating of pea and barley with Ni and Cd increased antioxidant capacity. In Cd treated YFP-peroxi Arabidopsis peroxules formed.

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# Insights into Sulfur Metabolism in the Halophyte *Tripolium pannonicum*: A Key to Understanding Tolerance Mechanisms to Salt and Cadmium Stress

In this study we analyse tight connection between sulfur metabolism and the response of the halophyte sea aster (*Tripolium pannonicum*) to salt and cadmium stress. We focused on the role of selected thiol-containing compounds (cysteine, glutathione, glutathione S-transferase – GST, and O-acetylserine – OAS) in stress tolerance. *T. pannonicum* showed good adaptation to both stressors, with no tissue dehydration and even growth stimulation at moderate NaCl concentrations. Salt stress strongly activated antioxidant defense mechanisms in *T. pannonicum*, as evidenced by the highest levels of total glutathione, phenolic compounds, DPPH radical scavenging activity, and the highest GSH/GSSG ratio. Glutathione dominated the thiol pool in NaCl-treated plants (88%), reaching up to 172 µM/g, highlighting its key role in salt stress response. In Cd-treated plants, GST activity doubled compared to control, suggesting a detoxification role. Interestingly, total protein content decreased under salinity, possibly indicating altered protein metabolism. Regarding phytochelators, PC4 prevailed under control and salt conditions, while PC2 dominated in cadmium-stressed plants (reached 11096 nm/g), highlighting its role in metal detoxification. In the next stages of our research on *Tripolium pannonicum*, we will investigate molecular and epigenetic responses to salt and cadmium stress, with a focus on their impact on thiol biosynthesis and sulfur metabolism.

## additional info

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### Poster 65

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# Comparison of the alterations in the root apex architecture of *Tilia cordata* Miller (Linden) growing on mining sludge after 6 and 12 weeks of exposition

The aim of the study was to show the sequence of alterations in the root apices of lime trees growing on mining sludge, contaminated with toxic elements (TMs) - in the perspective of using this tree species for phytoremediation. The nature and scale of alterations were compared in lime trees growing for 6 and 12 weeks in mining sludge. After 6 weeks, some disturbances were observed in the roots, but overall their architecture was not markedly different from that of control. We did not observe such severe malformations as those detected after 12 weeks, e.g. empty space where cells had collapsed or root tips without a root cap. Interesting alteration occurring after 6 weeks, in the several cell layers of the root cap, was a strong thickenings of the periclinal CWs, characterized by a high level of LM19 pectin epitope, able to bind the TMs ions. This alteration, not present in the control, was most likely a manifestation of the plant's defense strategy and could constitute an effective barrier limiting the penetration of TMs into the internal tissues of the root apex, especially the meristem. Manifestation of such a defense strategy suggests that lime trees exhibit promising phytoremediation potential.

## additional info

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## Plants and abiotic stresses

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# Dynamic interplay between m6A modification and the stress granules transcriptome during hypoxia and reoxygenation

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Stress granules (SGs) are non-membranous cytoplasmic structures that form in response to biotic or abiotic stress. Composed of proteins and poly(A) RNA, SGs serve as reservoirs for non-translating transcripts. Despite their widespread occurrence, their exact function and role in stress tolerance remain poorly understood. In this study, we show that two-zone SGs, previously undescribed in the literature, are formed in the roots of *L. angustifolius* under hypoxic stress. These structures consist of an outer ring enriched in poly(A) RNA and a central zone containing ribosomes. We observed distinct spatial distributions of mRNAs for stress-responsive and housekeeping genes within the zones of SGs. By inhibiting nucleocytoplasmic transport, we found that SGs are the primary source of cytoplasmic mRNA during the early stages of reoxygenation. The level of transcripts was sufficient to support cellular function during the initial hours following reoxygenation. Notably, under hypoxia, SGs were enriched with transcripts containing N6-methyladenosine (m6A) modification. Analysis using *A. thaliana* mutants with reduced m6A levels revealed a significant decrease in both the number of SGs and their poly(A) RNA content. These findings suggest that m6A is essential for SG formation and plays a critical role in maintaining mRNA stability and storage in these structures during hypoxic stress.

## *additional info*

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## Plants and abiotic stresses

### Poster 67

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# Growth and photosynthetic response of oat (*Avena sativa*) to phosphorus deficiency and struvite application

Phosphorus (P) is essential for optimal plant growth and development. P is a component of organic compounds important for metabolism, participates in intracellular signal transduction, regulates gene expression and enzyme activity, and is crucial for photosynthesis. However, in nature and in agricultural soils, Pi deficiency is common. Mineral phosphate fertilisers are used to meet the plant's high demand for P. These are produced from phosphate rock, a non-renewable resource. An alternative to traditional fertilisers is struvite, recovered from wastewater, characterised by slow nutrient release in soil. This study investigated the physiological and biochemical responses of new oat (*Avena sativa*) varieties, Gepard and Motto, grown under hydroponic conditions, in response to P deficiency and the application of struvite as a P source. Analyses of plant shoot and root growth parameters and water content were conducted. The value of the Nitrogen Balance Index, Pi content, and photosynthetic pigments were determined. In addition, the fluorescence of chlorophyll *a*, the activity of catalase, and the level of H<sub>2</sub>O<sub>2</sub> were measured. P deficiency negatively impacts plant growth and photosynthetic activity. Our results indicate that oat plants utilize phosphorus from struvite, but further studies are needed to fully evaluate the effectiveness of this approach.

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# Nanoplastic-induced abiotic stress disrupts pollen tube growth *in vitro*

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Abiotic stress induced by micro/nanoplastic (MP/NP) has a significant impact on living organisms, including plants. While the negative effects of MP/NP on soil biota and plant physiology are becoming obvious, there is no information on their possible impact on plant reproduction. It is known that NPs present in soil can penetrate into the roots and cause damage to plant cells, for example, structural defects in cell membranes and disturbed distribution of intracellular molecules. Furthermore, recent studies revealed that plant exposure on NP and foliar-applied of atmospheric nanoparticles has negative physiological effects. Sperm cells of angiosperms are immotile and require transport by the pollen tube to the embryo sac, where fertilization occurs. Efficient pollen tube growth is crucial in the multi-stage process of generative plant reproduction. Here we show that *in vitro* growing Petunia pollen tubes treated with NP exhibit multiple defects during elongation. They are significantly shorter, abnormally shaped, vacuolated, and often ruptured. NP-treated tubes also show disturbed distribution of cell wall polymers, vesicle transport and actin cytoskeleton organization. These results show for the first time that abiotic stress induced by NP disrupts pollen tube growth *in vitro* and consequently may have negative effect on plant reproduction and seed formation.

## *additional info*

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# *In situ* localization of cold-responsive genes related to aquaporins selected by RNA sequencing in sorghum roots

Climate change has forced the search for new sources of resistance among crop species to various abiotic stress factors. Sorghum, one of the most important cereal crops with many uses, including human food, animal feed and building materials is a suitable candidate for cultivation in areas with poor soil quality and frequent rainfall deficiencies. However, compared to other thermophilic species, such as maize, sorghum is more sensitive to low temperatures, which often occur in moderate climates of Europe. The water balance and its regulation by aquaporins, proteins involved in the transport of water and small molecules across cell membranes, are often discussed in the context of the search for mechanisms of the cold stress response in plants. In this study, we tested the hypothesis regarding the effect of low temperature on changes in the expression/localization of aquaporin transcripts in the roots of two sorghum lines differing in their chilling sensitivity. For this purpose, we performed *in situ* hybridization of selected genes based on RNA sequencing results. The observed changes in the labeling intensity of one of the tested aquaporin transcripts may indicate its possible role in the regulation of water uptake by sorghum roots under cold stress.

## *additional info*

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## Plants and abiotic stresses

### Poster 70

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# Phenotypic changes in *Arabidopsis thaliana* *lpcat* knockout mutants under cold and heat stress

Temperature stress can significantly impact plant growth and development. Acyl-CoA:lysophosphatidylcholine acyltransferases (LPCATs) are one of the enzymes that play a role in phosphatidylcholine remodeling, a critical process for maintaining membrane integrity under stress conditions. We investigated the physiological and metabolic consequences of knocking out *lpcat1* and *lpcat2* genes in *Arabidopsis thaliana*. Wild-type and mutant plants were grown in soil for 2 weeks or *in vitro* for 1 week under optimal conditions and then transferred to cold (~6°C) or heat conditions (35°C/25°C, day/night). Morphological observation of *in vitro* cultivation under standard and heat conditions showed that the roots of knockout lines developed slightly worse than wild-type, whereas this tendency was not observed under cold stress cultivation. The mutant lines grown *in vivo* were characterized by an altered rosette weight – increased under cold and decreased under heat. At the budding stage, lipidomic analysis and enzyme activity assays were conducted, including determination of the content and composition of membrane lipids, for which specific changes were detected. Additionally, we observed significant alteration in sugar content and pigments content between the mutant lines and the wild-type. Our results demonstrate a previously unknown role of the LPCAT enzyme in plant resilience to temperature stress.

## additional info

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## Plants and abiotic stresses

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# Photosynthetic Regulation and Drought Resilience Revealed by mRNA Metabolism Mutants in Barley

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Climate change, population growth, and increasingly frequent drought events threaten agricultural productivity. Improving drought tolerance in crops is therefore critical priority. Barley (*Hordeum vulgare*), the fourth most cultivated cereal worldwide, is a valuable model for studying stress adaptation in temperate cereals. We investigated the role of the Cap-Binding Complex (CBC), a key regulator of mRNA metabolism. Three barley mutants carrying changes in genes encoding CBC subunits - *hvcbp20.ab*, *hvcbp80.b*, and the double mutant *hvcbp20.ab/hvcbp80.b* were identified using TILLING method. Previous studies suggest that CBC mutations can enhance drought tolerance in various plant species. Drought stress was applied during the booting stage, a key period for spike development and yield determination. Physiological parameters (photosynthetic efficiency and gas exchange) and transcriptomic profiling revealed mutant-specific drought responses. Notably, CBC mutations modulated photosynthesis under both drought stress and optimal conditions, suggesting a broader role of CBC in regulating developmental and stress-responsive processes. These findings identified CBC as a promising target for enhancing drought resilience in cereals.

## *additional info*

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**Poster 72**

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# Deciphering the phasic changes of PM H<sup>+</sup>-ATPase under salt stress

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Plants respond to salt stress in root cells through a complex adaptive process characterized by the initial mitigation of Na<sup>+</sup> toxicity and subsequent growth recovery. The PM H<sup>+</sup>-ATPase is a vital protein that controls processes essential for Na<sup>+</sup> detoxification and cell elongation. Our research investigates the dynamic regulation of the PM H<sup>+</sup>-ATPase in *Arabidopsis thaliana* root cells over the course of a 24-hour salt stress response. We demonstrated that PM H<sup>+</sup>-ATPase activity undergoes phasic changes that most likely correlate with the different phases of salinity adaptation. Furthermore, we demonstrated that the PM H<sup>+</sup>-ATPase is regulated at the gene expression level during the first 24 hours of adaptation to salinity. In parallel, subcellular localization analysis using a high-end Lattice Lightsheet 7 microscope revealed dynamic changes in the PM H<sup>+</sup>-ATPase distribution within root cells, shifting its localization from the plasma membrane to the cytoplasm under salt treatment. These findings suggest that adjusting the subcellular localization of the PM H<sup>+</sup>-ATPase might act as a fast regulatory mechanism in the initial salt stress response. Future studies will explore the roles of brassinosteroids and vesicular trafficking in orchestrating this precise regulation of the PM H<sup>+</sup>-ATPase.

**Plants and abiotic stresses**

**Poster 73**

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# Uncovering the role of negative modulator of ABA-driven stomatal closure in barley

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Drought is a leading threat to crop productivity and improving plant responses to water deficit is a key agricultural priority. Rapid stomatal closure, driven by the hormone abscisic acid (ABA), helps plants conserve water, but the regulators that temper this response remain poorly defined. In barley, we explored the role of Cap-Binding Complex (CBC), in ABA-mediated stomatal control by comparing wild-type and CBC-deficient lines under ABA treatment and high vapor-pressure deficit. Barley mutants in genes encoding CBC subunits displayed altered stomatal conductance and distinct gene-expression profiles, highlighting the role of complex as a negative modulator of ABA signaling.

## *additional info*

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## Plants and abiotic stresses

### Poster 74

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# Hidden Hazards of Reused Wastewater: Physiological and Oxidative Effects of Common Pharmaceuticals on Rice

Rapid industrialization and wastewater reuse in agriculture to tackle water shortage for irrigation has led to the introduction of pharmaceutical pollutants in the environment. In this study, we have evaluated the impact of three common pharmaceuticals reported in wastewater, i.e., acetaminophen, sulfamethoxazole and estrone (E1) on rice (*Oryza sativa* L.) growth and physiology. For each, two concentrations were based on reported environmental concentration and the first concentration at which we found these pollutants affected seed germination. Rice plants treated with these pharmaceutical pollutants showed significantly lower photosynthetic rate at higher concentrations compared to control plants. The oxidative responses of plants to these pollutants and their underlying mechanisms were also investigated. Higher concentration of pollutants significantly increased peroxide level, malondialdehyde, proline content and ion leakage indicating membrane lipid peroxidation and cell membrane damage. In line with changes to reactive oxygen species, we also observed increases in antioxidant enzyme activities such as catalase, ascorbate peroxidase, superoxide dismutase, in plants treated with the compounds. Moreover, integrated biomarker response analysis revealed the level of oxidative stress created by each of the pollutants. These findings emphasize the need for sustainable wastewater management and further research on crop resilience to ensure global food security.

## *additional info*

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### Poster 75

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# Oxidative stress undermines longevity of European beech seeds (*Fagus sylvatica* L.)

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European beech (*Fagus sylvatica* L.) seeds display intermediate storage behavior and are highly susceptible to abiotic stress during storage, leading to rapid loss of viability and poor regeneration outcomes. This study integrates physiological, ultrastructural, and proteomic analyses to elucidate the cellular and molecular mechanisms underlying seed aging. Aged seeds exhibited diminished germination and seedling establishment, accompanied by substantial structural degradation, particularly in embryonic axes. Mitochondrial disorganization, including cristae disruption and membrane damage, was linked to oxidative stress, as evidenced by ROS accumulation, lipid peroxidation, and a decline in antioxidant enzyme activity (SOD, CAT, GR). Proteomic profiling revealed widespread changes in redox-related proteins, including thioredoxin h1 (Trx-h1), with 171 identified targets involved in protein folding, energy metabolism, calcium signaling, and epigenetic regulation. The presence of methyl-CpG-binding proteins among Trx-h1 targets suggests that redox-epigenetic interactions contribute to the process of aging. Collectively, these findings indicate that seed aging in beech is driven by redox imbalance, metabolic decline, and disrupted epigenetic homeostasis. This comprehensive view offers valuable insights into seed responses to abiotic stress and provides a foundation for developing strategies to improve storage and regeneration of forest reproductive material under changing environmental conditions.

## *additional info*

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## Plants and abiotic stresses

### Poster 76

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# Growth-Phase-Dependent Expression of Photosynthesis-Related Genes in Spring Triticale Seedlings under Drought and Recovery

Spring cereals offer a valuable alternative to winter varieties due to their shorter growing season and lack of vernalization requirements. However, they are highly susceptible to spring droughts that coincide with early seedling development. This study examined the responses of spring wheat (*Triticum aestivum* L., cv. Argus) seedlings at two developmental stages: 4-day-old (heterotrophic) and 6-day-old (autotrophic). Plants were subjected to mild (4-day) and severe (8-day) drought, followed by a 3-day rehydration period. Physiological traits (leaf relative water content, chlorophyll content, gas exchange) and expression of *psbA*, *petC*, *rbcl* and *RCA* were analyzed. Both seedling types showed drought-induced water loss, reduced photosynthesis, and growth inhibition. Autotrophic seedlings were more sensitive, with lower water content and chlorophyll degradation, but under mild drought they maintained higher gas exchange and increased *psbA*, *RCA1β*, and *RCA2β* expression. Under severe drought, gas exchange declined and *petC* was suppressed. Heterotrophic seedlings, though less active during mild drought, sustained gas exchange and upregulated *petC* and *rbcl* under severe drought. After rehydration, they recovered more effectively than autotrophic seedlings. These results highlight that even a two-day difference in drought onset leads to distinct physiological and molecular responses, emphasizing the importance of developmental stage in drought resilience.

**Plants and abiotic stresses**

**Poster 77**

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# Physiological and cellular responses of *Arabidopsis thaliana* plant to electromagnetic field exposure

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All living organisms on earth including plants, are subjected to the actions of natural and human-made magnetic fields (MFs). Industrial and technological advancements have significantly increased the artificial sources of electromagnetic fields (EMFs) and are often considered as additional silent stressors. Numerous reports highlight the potential of MFs for improved crop production by positively influencing seed germination and plant growth, development, and yield. However, the exact mechanisms of MFs action in plant cells is still poorly understood. Moreover, MFs affect different plant species differently, confirming the need for more basic research in this area. The aim of this study was to analyse the physiological and cellular responses of the model plant, *Arabidopsis thaliana* to treatment with an extremely low frequency (ELF, 50 Hz) electromagnetic field with a dominant magnetic or electric component, in the presence of salt and drought stress. Apart from growth parameters, several biochemical factors were analysed in *Arabidopsis* tissues: (1) selected phytohormones, (2) hydrogen peroxide, and (3) proline as an indicator of abiotic stress. The obtained results indicate significant impact of electromagnetic fields exposure on *Arabidopsis* physiology depending on the type of stressor applied.

## *additional info*

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**Plants and abiotic stresses**

**Poster 78**

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# Impact of salinity on PSII/LHCII phosphorylation in the halophyte *Mesembryanthemum crystallinum*

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Efficient regulation of photosynthetic electron transport is a key element of acclimation to adverse environmental conditions, as it allows optimal production of reducing power and ATP while limiting the formation of reactive oxygen species. Previously, we have demonstrated that acclimation to salinity in the model halophyte *Mesembryanthemum crystallinum* L. (Aizoaceae; common ice plant) is associated with a more reduced plastoquinone pool despite a bigger pool of open PSII reaction centers. Therefore, the aim of this study was to investigate how these redox changes in salt-acclimated plants affect PSII/LHCII phosphorylation and the organization of protein complexes in thylakoids. Western blot analyses revealed that, after 10 days of irrigation with a NaCl solution, the phosphorylation of the LHCBI, LHCB2 and D1 proteins increased under light and dark conditions. 77K chlorophyll fluorescence emission spectra showed that salinity promotes the association of LHCII with PSI, leading to a permanent State II. Furthermore, blue native polyacrylamide gel electrophoresis revealed partial disassembly of PSII supercomplexes. The changes in the phosphorylation and composition of thylakoid complexes are discussed in the context of acclimation to salinity.

**Plants and abiotic stresses**

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# Cadmium stress alters sugar metabolism and cell wall structure in rapeseed (*Brassica napus* L.) cell suspension culture

Cadmium (Cd) is a highly toxic heavy metal that significantly impairs plant growth and development, posing a substantial risk to global agriculture. Rapeseed (*Brassica napus* L.), a major oilseed crop in eastern Europe, is particularly susceptible to Cd toxicity. This study examines the physiological and structural responses of rapeseed cell suspension culture exposed to Cd at concentrations of 150 and 300  $\mu$ M for 48 hours. The activity of UDP-glucose pyrophosphorylase – an essential enzyme linking carbohydrate metabolism to cell wall biosynthesis – was investigated to elucidate its role in cellular adaptation to Cd-induced stress. Additionally, scanning electron microscopy (SEM) was employed to characterise the structural modifications of the cell wall under cadmium exposure. Results indicate modulations in enzyme activity accompanied by pronounced remodelling of the cell wall structure, reflecting an active cellular response to mitigate Cd toxicity. These findings enhance our understanding of heavy metal stress responses in plants and may have important implications for developing effective phytoremediation approaches.

## *additional info*

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## Plants and abiotic stresses

### Poster 80

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# Intra- and intergenerational plant stress memory - barley as a case study

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As sessile organisms, plants developed complex adaptive mechanisms to cope with environmental stresses. Plant stress memory refers to the ability of plants to retain information about past environmental stimuli and utilize it to enhance responses upon re-exposure. In this study, we aimed to unfold some aspects of the intragenerational memory that helps plants respond swiftly to immediate stressors within the same generation and intergenerational memory that ensures the offspring inherit a degree of resilience, enhancing their chances of survival. To study different aspects of plant memory, we have applied the Dark-Induced Leaf Senescence (DILS) model, which leads to premature senescence in barley leaves, including reduced photosynthetic efficiency. After treatment and following the recovery phase, plants were re-exposed to DILS within the same (intragenerational memory) or next generations (intergenerational memory). Changes in the dynamic of chlorophyll fluorescence in the response to secondary DILS were more consistent when compared to the primary DILS. These varied responses indicate the existence of intragenerational memory. Moreover, the longest dark-treatment decreased capacity to recover in subsequent generations (F1, F2) of barley plants exposed to DILS. Therefore, parents exposed to long-term stress can, through memory transmission, produce a generations that are not resistant to the same stress.

## *additional info*

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## Plants and abiotic stresses

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# Phosphorylation as a potential mechanism regulating cis-prenyltransferase activity during the biosynthesis of polyprenols in *Arabidopsis*

Leaf polyprenols are accumulated predominantly in thylakoid membranes, where they play a role in modulating membrane dynamics, thereby influencing photosynthetic efficiency. Furthermore, they are implicated in plant stress responses. The biosynthesis of polyprenols by cis-prenyltransferases (CPTs) is well documented, but the regulatory mechanisms governing CPT activity remain largely unknown. In *Arabidopsis thaliana*, plastidial polyprenols are synthesized by CPT7. Exposure of plants to elevated temperatures (38°C) strongly induces CPT7 expression and its lipid products accumulation. The lack of these compounds in the *cpt7* deletion mutant is critical for plant survival under heat stress. Interestingly, the expression of SnRK2.3 kinase is also significantly increased under these stress conditions. Moreover, we found that CPT7 is phosphorylated by SnRK2 kinases as shown by *in vitro* kinase assays and mass spectrometry analysis of the native CPT7p. The *snrk2* mutants show reduced CPT7 expression and, in parallel, a significant increase in polyprenol levels in comparison to wild-type plants. Based on these results we hypothesize that impaired phosphorylation leads to the activation of the CPT7 protein, resulting in enhanced polyprenol accumulation and downregulated CPT7 expression, due to a negative feedback mechanism. Collectively, this data suggests that phosphorylation of CPT7 by SnRK2 kinases may negatively regulate its activity.

## additional info

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# Mechanisms Underlying Salt Stress Interference with Chloroplast Movement

Light-induced chloroplast rearrangements are an adaptive mechanism for optimizing light use. These movements are also influenced by environmental factors beyond light, such as temperature. Here, we examine the effect of high salt stress on chloroplast movement. Chloroplast movements are inhibited by NaCl in a dose-dependent manner. Notably, this inhibition is significantly reduced in the *snrk2.1/4/5/10* mutant lacking group I SNF1-related kinase 2 (SnRK2) – plant-specific kinases that regulate responses to environmental stresses such as salinity and drought. Phosphoproteomic studies identified several chloroplast movement-related proteins as potential SnRK2 substrates. We focused on two: THRUMIN1 (TRM1) and PLASTID MOVEMENT IMPAIRED 1 (PMI1). *In vitro* assays confirmed that SnRK2 phosphorylates both proteins, and phosphorylation sites were mapped. Protein–protein interactions were also validated in planta. For TRM1, transgenic lines expressing a phosphorylation-deficient mutant showed reduced ability to rescue the *trm1* phenotype compared to wild-type TRM1, suggesting that SnRK2-mediated phosphorylation supports chloroplast movement under salt stress. We analyzed the subcellular localization of PMI1 and TRM1 and found that both proteins relocate to punctate structures under salt stress. These structures partially colocalize with FM4-64 dye, suggesting they are endocytic vesicles. Thus, endocytosis of movement-related proteins may contribute to their functional impairment during salt stress.

## *additional info*

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## Plants and abiotic stresses

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# The modification effect of blue, red and far-red radiation ratio on lettuce morphology and composition

Presented study aimed to examine the effect of light spectrum of glass containing red luminophore on crop characteristics, including morpho-anatomical features and composition. Plant material comprised two lettuce types: butterhead and iceberg cultivated in greenhouses covered with: transparent glass (control) and glass containing red luminophore (red). Red luminophore used, changed sunlight spectrum providing an adequate blue:red light ratio, while decreased red:far-red radiation ratio. Alterations were observed in head dimensions, morphology and leaf mesophyll structure of plants from red greenhouse. Butterhead lettuce plants exhibited unaltered head area under tested conditions, but displayed reduction in accumulated sugars and amino acids, resulting in decline in dry matter content. Conversely, increase in soluble and insoluble sugars and amino acids content and no change in nitrate content was observed in iceberg lettuce. However, growth intensity of iceberg lettuce decreased, while its dry matter content increased. Moreover, phenols and vitamin C concentration was lower in iceberg lettuce than in butterhead one. In red greenhouse, phenolic content declined in both lettuce type but vitamin C levels reduced in butterhead one and remained unchanged in iceberg one. Variation in crop characters in lettuce cultivated in red greenhouse depended on tested lettuce type, with notable alterations in iceberg lettuce.

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# Impact of Gadolinium on Biochemical Parameters in *Chlamydomonas reinhardtii*

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Gadolinium (Gd) is a rare earth element (REE) extensively used in modern technologies, particularly in medical imaging. Its increasing detection in aquatic environments raises concerns, yet few studies have explored its presence and effects on aquatic plants. Gadolinium accumulation poses a potential threat to aquatic organisms, especially microalgae, which form the foundation of aquatic food webs and play a crucial role in primary production and biogeochemical cycling. Despite these concerns, the ecotoxicological impact of gadolinium on microalgal physiology remains poorly understood. In this study, we investigated the effects of gadolinium exposure on the green microalga *Chlamydomonas reinhardtii*. We evaluated key biochemical parameters, including cell density, total carbohydrate and protein content, and photosynthetic pigment concentrations, under varying gadolinium concentrations and exposure durations. The results revealed dose-dependent metabolic alterations in the algae. Notably, high gadolinium concentrations inhibited cell growth and disrupted macromolecule synthesis, indicating cellular stress. Changes in pigment levels further suggested possible interference with photosynthetic processes. Our findings provide new insights into the sublethal effects of gadolinium on microalgae and underscore the need for further research on the ecological consequences of REE contamination in aquatic ecosystems.

**Plants and abiotic stresses**

**Poster 85**

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# Cyclic voltammetry as a method for determining the viability of seeds

Effective techniques for assessing seed viability are essential in agronomy and forestry. While some seeds can last thousands of years, others only survive for weeks, and all seeds eventually degrade over time in storage. One of the primary causes of seed aging is the presence of reactive oxygen species (ROS) and imbalances in antioxidants. Therefore, developing reliable methods to evaluate the antioxidant capacity of stored seeds is essential. This study investigates the application of cyclic voltammetry (CV) using a glassy carbon electrode to analyze the antioxidant profile in aging *Acer saccharinum* L. seeds. CV is a flexible electrochemical technique that does not require redox-active reagents, enabling the assessment of total antioxidant capacity based on their electrochemical behaviour. We tracked oxidative stress and antioxidant depletion by measuring ROS levels and quantifying antioxidants using both CUPRAC-BCA and CV. Cyclic voltammetry proved to be a more dependable method for evaluating total antioxidant capacity, demonstrating high correlations with seed viability ( $R = 0.92$ ;  $p \leq 0.01$ ). In conclusion, we established a strong link between CV results, antioxidant capacity, and seed viability, indicating that electrochemical techniques can efficiently assess seed viability before germination with an accuracy of 92%.

## additional info

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## Plants and abiotic stresses

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# Immunolocalization of aquaporins in the roots of maize plants treated with low temperature

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Various stress factors directly or indirectly affect plant water management. Low temperature stress leads to a reduction in water potential in different organs of most plants, especially in chilling-sensitive species such as maize. This can result in e.g. reduced root hydraulic conductivity and sap flow, causing distinct symptoms of water stress in shoots. It is clear that changes in water potential are controlled by aquaporins; the largest subfamily of plant aquaporins – PIP proteins (Plasma membrane Intrinsic Proteins) – is divided into two subgroups – PIP1 and PIP2, where PIP2 proteins typically exhibiting higher water conductance activity. In this work, we present the changes in the intensity of labelling of aquaporins in the roots of cold-treated plants of two maize varieties differentiated for chilling-sensitivity. For immunolocalization of aquaporins (PIPs), with visualization under a transmission electron microscope (TEM), root samples were collected from three experimental variants (control: non-chilled plants and chilled plants for 1 and 3 days) and were prepared according to standard procedures. The observed changes in aquaporin labeling in the membrane of root cells in maize lines may indicate the involvement of the tested PIP forms in the proper water balance maintenance under low temperature stress conditions.

## *additional info*

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## **Plants and abiotic stresses**

### **Poster 87**

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# The effect of polystyrene nanoplastics on the distribution of mitochondria and chloroplasts in the mesophyll cells of *Lemna trisulca* L.

Plastic pollution in the natural environment is one of the greatest challenges facing humanity in the 21st century. It becomes particularly hazardous to living organisms when, under the influence of biotic and abiotic factors, is degraded into nanoplastic. So far, no data have yet been presented on how polystyrene nanoparticles affect the distribution of mitochondria and chloroplasts in the mesophyll cells of flowering plants. To answer this question, we analysed the localisation of mitochondria and chloroplasts in *Lemna trisulca* L. frond cells treated with polystyrene nanoplastics at concentrations of 0 (control), 25, 50, 100, 150 mg/l for 30 days. Before observations plants were incubated for 12 h in darkness. In plants exposed to nanoplastics, clusters of mitochondria and chloroplasts were observed near the anticlinal cell walls, which were not observed in the control variant. In the variants of plants treated with nanoplastics, a significantly higher percentage of mitochondria indirectly adjacent to chloroplasts was observed. Furthermore, they moved closer to the anticlinal cell walls than those observed in the control group. The formation of clusters and changes in the distribution of mitochondria may be an indicator of stress caused by the presence of polystyrene nanoplastics.

## additional info

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## Plants and abiotic stresses

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# Light-Triggered Grana Dynamics: A Three-Phase Model of Early Photosynthetic Regulation

Understanding the dynamics of the thylakoid membranes during dark–light transitions is critical for both basic photosynthetic research and crop optimization. Here, we elucidate the temporal sequence and functional significance of light-induced thylakoid structural changes in various plant species. Employing a multi-modal strategy – transmission electron microscopy, confocal microscopy with 3D reconstruction, and small-angle neutron scattering – combined with spectroscopic and electrophoretic analyses, we characterized membrane remodeling under controlled illumination. A comprehensive meta-analysis of existing ultrastructural studies further resolved longstanding discrepancies over thylakoid shrinkage versus expansion. Our results reveal a universal three-phase response: (1) a rapid, transient shrinkage that modulates the cyclic/linear electron transport ratio to afford immediate photoprotection; (2) a subsequent expansion phase; and (3) a relaxation back to the dark-state equilibrium. Furthermore, we demonstrate that prior light acclimation profoundly influences response kinetics: plants grown under controlled light undergo structural adaptations more rapidly than glasshouse-acclimated ones. This triphasic model replaces the traditional binary framework of thylakoid dynamics, providing a unified explanation for previously conflicting observations. By linking structural plasticity to functional photoprotection, our findings offer new insights into the temporal regulation of photosynthesis and suggest strategies for engineering improved light resilience in crop species.

**Plants and abiotic stresses**

**Poster 89**

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# RNAi based biopesticide for sustainable crop protection: Improving efficacy and delivery of dsRNA

RNA interference (RNAi) is a promising strategy for sustainable pest control through sequence-specific gene silencing using double-stranded RNA (dsRNA). However, challenges remain regarding dsRNA stability and large-scale delivery methods. This research aims to develop a biopesticide with enhanced RNAi efficacy by improving dsRNA stability through nanoparticle-mediated delivery and to evaluate the systemic movement of dsRNA in both plant and insect systems via uptake and translocation mechanisms. As a proof of concept, the small conductance calcium-activated potassium channel gene (TmSK) in *Tenebrio molitor* was targeted. TmSK was expressed across all life stages, with the highest expression in the head. Injection of 2000 ng dsRNA into larvae (0.008% of the body mass) resulted in a 15.0% and 10.0% reduction in TmSK mRNA levels at 2 and 7 days post-treatment, respectively and reduced the survivability by 33.3% at 15 days. Subsequently, fluorescence microscopy will be used to track the distribution of the fluorescently labelled TmSK dsRNA-nanoparticle formulation within the leaf tissue and vascular system of pumpkin plants, and their fate following ingestion by *T. molitor*. A biosafety assessment will also be conducted using bumble bees to evaluate potential non-target effects, supporting this method as a holistic and sustainable approach to crop protection.

## *additional info*

The research reported here was funded by the Commonwealth Scholarship Commission and the Foreign, Commonwealth and Development Office in the UK.

## Plant biotic interactions

### Poster 90

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# Exploring a New Redox Signal in Potato Immunity against *Phytophthora infestans*

The cellular oxidation-reduction reactions are sources of nitric oxide ( $\bullet$ NO) conversion to other reactive nitrogen species, which can affect biological systems leading to different physiological effects. Nitroxyl ( $\text{HNO}/\text{NO}^-$ , also named azanone), a one-electron reduced and protonated congener of  $\bullet$ NO presents highly complex chemistry. This reactivity makes it a perfect candidate for a signaling molecule. Recently, the endogenous production of HNO in *Arabidopsis* was reported, suggesting a novel regulatory or signaling role in plants. In the present study, we showed that the pathogen attack promotes not only  $\bullet$ NO but also HNO formation in host cells. Using electrochemical microsensors measuring HNO and  $\bullet$ NO concentration up to low nanomolar levels in real-time, we detected that inoculation of potato (*Solanum tuberosum* L.) leaves with *Phytophthora infestans* (Mont.) de Bary resulted in an early HNO and  $\bullet$ NO generation. To recognize the redox environment of potato cells attacked by *P. infestans*, S-nitrosothiols and total antioxidant capacity (TAC) were measured over time. Importantly, TAC can assess cellular redox status, providing a comprehensive evaluation of non-enzymatic antioxidant activity, that encompasses the collective action of all antioxidants within a given matrix. The results allow us to estimate the relative  $\bullet$ NO/HNO interplay during the potato – Avr/vr *P. infestans* interaction.

## additional info

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## Plant biotic interactions

### Poster 91

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# Hymenoscyphus fraxineus Exopolysaccharides Modulate Plant Cell Wall Integrity and Defense Signaling in Ash and Arabidopsis

Ash dieback, caused by the invasive fungal pathogen *Hymenoscyphus fraxineus*, poses a major threat to ash tree populations in Europe. Understanding how plants respond to fungal infection is critical for uncovering resistance mechanisms. This study investigated the effects of fungal exopolysaccharides (EPS) on cell wall integrity and defense responses in *Fraxinus excelsior* and *Arabidopsis thaliana*. We found that EPS extracted from *H. fraxineus* contain mixed-linkage glucans and significantly trigger the expression of defense-related genes. However, this induction was markedly reduced in xyloglucan-deficient *Arabidopsis* mutants, suggesting that xyloglucan, a key structural component of the plant cell wall, plays an important role in mediating immune responses. Cell wall compositional analysis revealed significant alterations in xyloglucan structure and other components, including cellulose and glucose, following EPS treatment. In addition, EPS induced oxidative stress, as evidenced by elevated ROS levels and the subsequent deposition of callose, a  $\beta$ -1,3-glucan, at the cell wall and plasmodesmata. Defense signaling analysis identified the jasmonic acid/ethylene and salicylic acid pathways as key regulators of fungal glycosyl hydrolase gene expression, linking hormone signaling to pathogen interaction and carbohydrate metabolism. Altogether, this study provides valuable insights into plant defense strategies and may inform future approaches for enhancing resistance to fungal pathogens.

## additional info

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## Plant biotic interactions

### Poster 92

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# Investigative study of the novel members of the CYP81F enzyme family from Brassicaceae plants

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Glucosinolates are sulfur-containing defense compounds in Brassicales order, classified into aliphatic, aromatic or indole glucosinolates. Indol-3-ylmethyl glucosinolate (I3G) is modified by four *A. thaliana* CYP81F monooxygenases. CYP81F1, CYP81F2 and CYP81F3 mediates formation of 4OH I3G, while CYP81F4 functions in biosynthesis of 1OH I3G, which are subsequently methoxylated by IG O-methyltransferases. Our recent study revealed, species closely related with *A. thaliana*, such as of *Capsella*, *Camelina* and *Neslia* genera, lost CYP81F2 and CYP81F4, but acquired CYP81F5 and CYP81F6 with unknown functions. In this study, we performed analysis of available genomic sequences of Brassicaceae species to identify CYP81F orthologs. Putative CYP81F5 orthologs are found in *Thlaspi arvense*, and in two species from Isatideae tribe. Moreover, we found CYP81F6 orthologs in *Arabis alpina*, *Boechera stricta* and *Malcolmia maritima*. Additionally, we investigated if and at which positions CYP81F5 and CYP81F6 from *Capsella rubella* hydroxylate I3G in planta. We expressed these enzymes in *cyp81f2/f4 A. thaliana*, deficient in 1MI3G biosynthesis and accumulates strongly reduced amounts of 4OH I3G and 4MI3G in leaves, but hyper-accumulates the CYP81Fs substrate, I3G. Metabolic analysis of generated transgenic lines indicated CrCYP81F5 is able to hydroxylate I3G to produce 4OH I3G. It has been also concluded that CrCYP81F6 is not capable of modifying I3G.

## additional info

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## Plant biotic interactions

### Poster 93

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# The role of peroxidases in the response of barley to salinity and mite *Aceria tosichella* infestation

Peroxidases play a crucial role in cellular defense mechanisms under oxidative stress by utilizing hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as an electron acceptor. This study examined changes in gene expression and activity of various peroxidases (general activity (POD), guaiacol (GOPX), ascorbate (APX), and glutathione (GPX)) in barley under salinity and *Aceria tosichella* (wheat curl mite, WCM) infestation. Plants were divided into 6 experimental groups: control, treated with 50 mM NaCl, 100 mM NaCl, inoculated with WCM, WCM+50 mM NaCl, and WCM+100 mM NaCl. A similar expression pattern was observed for all tested genes, and an increase in the relative expression level was noted in 100 mM NaCl and 100 mM NaCl+WCM. In turn, the enzymatic activity of individual peroxidases was characterized by a diverse pattern. POD activity increased in all tested combinations (except 50 mM NaCl), APX activity in 100 mM NaCl+WCM, GPX activity in all combinations, and GOPX activity in 50 mM NaCl and 100 mM NaCl+WCM. The observed changes indicate a notable adjustment of the antioxidant system, finely tuned to the type and severity of the experienced stress.

## Plant biotic interactions

### Poster 94

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# More stress, more mess: Bacterial effector interaction with plant stress granules

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Climate change triggers the emergence of novel pathogens and unprecedented outbreaks of plant diseases, threatening global food security. Plants counteract environmental stress through sophisticated, adaptive mechanisms, including the formation of biomolecular condensates such as stress granules (SGs). These dynamic structures sequester translationally stalled mRNA and RNA-binding proteins within, “hiding” essential molecules from degradation during stress conditions. Despite their critical role in stress responses, the function of SGs in plant-pathogen interactions remains poorly understood. The presented research investigates whether *Pseudomonas savastanoi* pv. *phaseolicola* effectors target SGs to promote infection, or SGs contribute rather to plant immunity? Bioinformatic screening identified five candidate effectors: PphHopAU1, PphHopG1, PphHopX1, PphHopD1, and PphHopAW1, containing structures potentially involved in association with SGs. Confocal microscopy revealed that under heat stress, three effectors, namely PphHopAU1, PphHopG1, and PphHopX1, exhibited characteristic patchy patterns and co-localised with UBP1b, a known stress granule marker. We have successfully developed a method for the isolation and purification of intact SGs using anti-GFP antibodies and Dynabeads protein A. This will enable future analysis of SGs composition through RNA sequencing and mass spectrometry. These results establish the foundation for analysing how bacterial effectors interplay with plant SGs.

**Plant biotic interactions**

**Poster 95**

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# A broad spectrum of *Brassica* species responses to *Alternaria brassicicola* infection

Interactions of *Brassica* species, such as *B. oleracea*, *B. juncea*, or *B. napus*, with the model necrotrophic fungus *Alternaria brassicicola* are considered susceptible as revealed at macroscopic, metabolic, ultrastructural, and transcriptomic levels. The most downregulated process in these interactions was photosynthesis, as evidenced by chlorosis and necrosis, chloroplast degradation, a decrease in chlorophyll content, and the downregulation of photosynthesis-related genes. However, a few upregulated processes correlated with fungal development were found in the plant response at the early stages of infection. First, the activation of a signaling cascade, including the upregulation of RLK7 and WRKY33 genes, began with the generation of reactive oxygen species (ROS) and subsequent activation of the antioxidant system. Interestingly, diverse patterns of enzymatic and non-enzymatic antioxidants were triggered in each *Brassica* species in response to *A. brassicicola* infection. Next, the contents of individual glucosinolates and sugars increased in a cultivar-dependent manner and correlated with its level of resistance to *A. brassicicola* infection. Moreover, changes in jasmonic acid content and jasmonate-responsive gene expression, such as the upregulation of PDF1.2 and the downregulation of LOX2, were observed. All these events indicate that even a successful invasion of a susceptible host by the fungus releases temporary defense responses.

## additional info

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## Plant biotic interactions

### Poster 96

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# First Comprehensive Analysis of Non-coding RNA Structures in Oat Using Nanopore Technology: Insights into Biotic Stress Responses

Non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), are fundamental regulators of gene expression involved in plant development and stress responses. Growing evidence demonstrates their critical roles in plant defense mechanisms against biotic stresses, particularly fungal pathogens. While ncRNA functions have been extensively studied in model crops like wheat and barley, comprehensive analysis in oat (*Avena sativa* L.) remains largely unexplored. Research on wheat has revealed differential expression of lncRNAs during *Blumeria* and *Puccinia* infections, with some functioning as miRNA precursors or direct regulators of defense pathways. Similarly, studies in other cereals have identified ncRNA-mediated regulation of immune responses, suggesting evolutionary conservation of these mechanisms across related cereal species. Despite the agricultural importance of oat and its susceptibility to powdery mildew (*Blumeria graminis*) and rust (*Puccinia* spp.), no comprehensive ncRNA analysis has been conducted for this crop. This knowledge gap is particularly significant given oat's complex polyploid genome structure. This ongoing research presents the first comprehensive analysis of ncRNA structures in oat using advanced sequencing technologies, including Oxford Nanopore sequencing. Our study aims to identify and characterize miRNA and lncRNA responses to *Blumeria* and *Puccinia* infections, contributing to understanding molecular resistance mechanisms in this important cereal crop.

## additional info

This study was supported by National Science Centre (Poland) in the frame of SONATA grant (2023/51/D/NZ2/02115) entitled "Analysis of the influence and identification of non-coding RNA (miRNA and lncRNA) sequences in transcriptomes of common oat (*Avena sativa* L.) plants induced by fungal infection with *Blumeria* and *Puccinia* genera."

## Plant biotic interactions

### Poster 97

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# PME and PME1 as a tandem involved in Arabidopsis response to *Turnip mosaic virus*

Pectins in the cell wall have many important functions, the main pectin components, homogalacturonans (HGs), were postulated as defensive molecules in plants during infection and have been linked to disease resistance. The most important in HG methyl/demethylesterification are tuned by enzymes-PME [EC 3.1.1.11] and proteinaceous inhibitors (PMEIs), with a diverse effect on pectin biosynthesis. PME and PME1 primarily participate in stress signaling, and defense immunity. Therefore, our studies aimed to examine the regulation of selected *A. thaliana* pectin metabolism components in susceptible rbohD-TuMV and Col-0-TuMV as well as resistance rbohF-TuMV and rbohD/F-TuMV reactions. Susceptible reactions were displayed with upregulation of AtPME3, which was also confirmed by induction of PME3 deposition. Our results revealed the highest PME activity in rbohD-TuMV, as well as significant domination of low/non-methylesterified HGs and general decrease in cell wall methylesters. Conversely, cell wall rebuilding in the resistance response of rbohF and rbohD/F to TuMV was associated with dynamic induction of AtPME12 and AtPME13, which was additionally confirmed by significant induction of the deposition of PME12, PME13. Therefore, PME12 and PME13 highly methylesterified HGs were actively distributed while participating in rbohF and rbohD/F defense response and cell wall rebuilding in rboh-TuMV pathosystem.

## additional info

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## Plant biotic interactions

### Poster 98

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# INVESTIGATING THE ANTIMICROBIAL POTENTIAL OF ENDOPHYTES TO PROTECT KHAT (*CATHA EDULIS*) FROM PATHOGENS

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Background: Plants can control and appose stress on their bacterial phytopathogen in different ways. We investigate the antimicrobial potential of Khat leaves extracts and its endophytes. Khat (*Catha edulis*) is a perennial shrub well known for its psychological activity, causing a mildly stimulating euphoric, amphetaminic like effect. In addition, khat also played important roles in traditional folklore medicine, to treat common illnesses such as stomachache, diarrhea and pneumonia. Methods: We extracted khat leaves, using either water, or organic solvents. The antimicrobial tests were performed using the Kirby-Bauer Disk Diffusion Susceptibility test against environmental, human pathogens and phytopathogens bacteria. Endophytes isolated from Khat leaves were determined using 16S rDNA gene sequencing. Results: All tested solvents yielded extracts with antimicrobial properties, but methanol and acetone extracts displayed the strongest antibacterial effects. Heating methanolic extracts to 100°C did not abolish the antimicrobial activity. Eight out of 18 endophytes isolate from Khat leaves showed antimicrobial activity against environmental, human pathogens and phytopathogens bacteria. Conclusions: khat leaves possess antimicrobial activity, that resist heating to 100°C. Antibacterial compounds, readily extracted into methanol. khat leaves contains endophytes resist Khat antibacterial activity, possess antimicrobial effect by their own that may contribute to the antimicrobial activity of the plants.

**Plant biotic interactions**

**Poster 99**

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# RICHFUN - enrichment and decoding of fungal biosynthetic clusters in low-abundant, plant-associated mycobiomes

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"To what extent different plant-derived environments impact profiles of fungal biosynthetic potential?" Correct answer is dependent on the means to correctly identify genetic determinants of biosynthetic potential. Focusing on the environmental DNA, assuming knowledge of the genetic determinants of chemotype (key biosynthetic genes), this can be distilled to two separate goals. First, to compile a frame of reference upon which environmental DNA (eDNA) sequences can be classified and interpreted. Second, to devise an efficient way to amplify the mycobiome's biosynthetic component of the eDNA, with a particular care of minimising contamination with bacterial eDNA. Most extant studies prioritize taxonomic (meta)barcoding and not quantification of variation for the genetic determinants of chemotype such as toxigenicity or pigment biosynthesis. We present the theoretical background and preliminary results aimed at novel hybridisation-based approach, combining next generation sequencing and phylogenomic annotation in order to fill the niche between barcoding and shotgun eDNA sequencing for exploration of biosynthetic potentials present in extant mycobiomes: micro-environments associated with diverse, agriculturally important plants including both monocots (*Miscanthus* and *Festuca* spp.) and dicots (*Lupinus* and *Solanum* genera).

## additional info

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## Plant biotic interactions

### Poster 100

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# Tolerance and stress response in the $\omega$ -gliadin-free winter wheat line under *Fusarium culmorum* pressure

*Fusarium culmorum* is a common fungal pathogen responsible for significant yield losses and quality reduction in cereals due to root rot, head blight, and mycotoxin contamination. This study evaluated the physiological responses and tolerance levels of three winter wheat genotypes: Persona, Symetria, and the hypoallergenic line wasko.gl– to *F. culmorum* infection. The wasko.gl– genotype, which lacks  $\omega$ -gliadin fractions associated with severe gluten-induced allergies, was of particular interest. Plants were assessed 8 days post-inoculation for selected chlorophyll fluorescence parameters (Fv/Fm, Fv/F0, PI, RC/ABS), leaf reflectance indexes, pigment content (chlorophylls, flavonols, and the NBI index), catalase (CAT) activity and PrxQ levels. FT-Raman spectroscopy was used to analyze biochemical changes in leaf tissues and in flour samples obtained after the regeneration of infected plants. The results indicated that wasko.gl– winter wheat line exhibited the highest tolerance to infection, with minimal pigment loss, reduced physiological damage, and enhanced regeneration capacity compared to the other genotypes. Genotype and treatment interaction significantly affected photosynthetic efficiency, while flour analysis revealed only minor changes in starch content and disulfide bond conformation in wasko.gl–. These findings suggest that the hypoallergenic wheat line may offer both health benefits and improved resistance to *F. culmorum*-induced stress.

## additional info

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## Plant biotic interactions

### Poster 101

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# Investigation of the mechanical properties and *Potato Virus Y* (PVY) resistance of dihaploid potato plant genotypes

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In plants, growth and morphogenesis depend on the interaction of genetic networks, cell signaling, and mechanical forces. Turgor pressure within cells stretches the cell wall elastically, and under the influence of wall modifiers, this tension causes the wall to expand through creep. Growth depends on the product of extensibility factors and turgor pressure exceeding the yield point. The selected diploid potato genotypes with varying levels of genetic resistance to PVY were treated with *Potato virus Y*. Three days post-inoculation, leaves were stretched using a micro-extensometer with low-cost optical tracking and controlled by MorphoRobotX software. The significance of this experiment lies in its interdisciplinary approach, combining plant virology and genetics to explore a new tool that measures biomechanical properties in potato plants, a crop of critical global importance. Additionally, the findings could have practical applications in agriculture, particularly in the development of new equipment for biophysical examinations.

## *additional info*

The research internship was funded by internal financial resources of Plant Breeding and Acclimatization Institute - National Research Institute (Poland) and Majda Lab (Switzerland).

## Plant biotic interactions

### Poster 102

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# Sterol profile remodeling and increased triterpenoid accumulation induced by fungal infection in *Quercus robur* leaves

Triterpenoids and steroids are often considered plant metabolites involved in various defense mechanisms against biotic stresses, such as pathogen attacks. The aim of this study was to investigate changes in the content of these compounds in oak (*Quercus robur*) leaves infected by the fungal pathogen *Erysiphe alphitoides*, which causes powdery mildew - one of the most common diseases affecting oaks. Gas chromatography-mass spectrometry (GC-MS) analysis revealed that in extracts obtained from infected leaves, the total content of steroids decreased by approximately 18% compared to healthy leaves. The sterol profile was altered due to a reduction in sitosterol content (by up to 27%), accompanied by a twofold increase in stigmasterol. Such characteristic changes in the ratio of sitosterol to stigmasterol often occur during the rearrangement of plant membranes in response to various stresses. Furthermore, the total content of triterpenoids was up to 2.5-fold higher in infected leaves than in healthy ones, including a particularly notable eightfold increase in the level of triterpenoid acids. The results of this study indicate that infection with *E. alphitoides* triggers metabolic alterations in *Q. robur* leaves, leading to increased biosynthesis of triterpenoids alongside a slight reduction in sterol content.

## additional info

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## Plant biotic interactions

### Poster 103

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# The role of selected transporting pathways in wood formation

Intercellular transport plays an important role in many developmental processes in plants. Secondary xylem (wood) is a conductive tissue characterised by a heterogeneous character, as it is composed of both dead and living elements. Therefore, studying intercellular communication in wood is particularly difficult and challenging. Given the difficulties associated with studying trees, *Arabidopsis thaliana* serves as a model also in wood research. Our current research focuses on the role of clathrin-mediated endocytosis (CME) in the uptake of molecules from dead conductive elements to living vessel associated cells (VACs) and symplasmic transport between neighboring living parenchyma cells during secondary wood formation and functioning. In our wood project, we take advantage of various transgenic *Arabidopsis* plants (with constantly and inducibly increased/decreased callose levels and hampered CME), and we employ methods routinely used in plant developmental biology (e.g. paraffine sections), transport studies (e.g. immunolocalisation), and molecular biology (e.g. qRT-PCR). Such experimental approach will enable more straightforward verification of working hypotheses.

## additional info

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## Short- and long-distance communication in plants

### Poster 104

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# The preliminary research on the effect of acoustic waves on *Cannabis sativa* L. metabolome

Plants are able to emit and detect sound waves, and respond to them through alterations in gene expression, signalling and cellular metabolism. *Cannabis sativa* L. could be a good model for studying this phenomena due to abundant secondary metabolism highly concentrated in glandular trichomes. The study focused on modifications induced by acoustic waves in one-month-old plants of cv. Enectalina. Pure tone recordings of 2kHz and 200Hz frequency were played continuously at 80 dB. Leaf samples were collected after 0, 1, 3, 6 and 9 days of treatment and analyzed using HPLC-MS/MS to measure the level of selected phytohormones, phenolic compounds and flavonoids. The obtained results indicate that both the duration of the stimulus and acoustic wave frequency significantly affected the plants. Increased contents of some phytohormones (ABA, JA, SA and IAA) and flavonoids (i.a. rutin, quercetin, isoquercetin, naringenin, vitexin, luteolin) were observed after the 1st to 3rd day especially after the treatment with 2kHz frequency. Our initial results indicate that both the duration and frequency of acoustic stimulus affects plant metabolism. These findings may have important implications on regulating plant defensive system, as well as in secondary metabolite production. Further studies are needed to understand this phenomenon.

## additional info

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## Short- and long-distance communication in plants

### Poster 105

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# UVSSA: A DNA repair protein with a connection to cyclic nucleotides metabolism

The presence of cyclic nucleotides (cNMPs) in plants has been controversial for many years due to their low concentrations in cells and the absence of homologs known from animal or bacterial systems. Recent advances in detection methods have confirmed the existence of cNMPs in plant cells and also led to the identification of proteins involved in their metabolism – cyclases responsible for cNMP synthesis and phosphodiesterases (PDEs) responsible for their degradation. These findings support the growing recognition of cNMPs as signaling molecules involved in a wide range of physiological processes such as ion homeostasis, hormone-related pathways, stress responses, immunity, and light-regulated processes such as phytochrome-mediated flowering. Additionally, short-term UV radiation has been shown to induce intracellular cNMP-level changes, implicating them in environmental stress responses. The UVSSA (AT3G61800) protein has been identified as having both a PDE domain and a guanylate cyclase (GC) domain using bioinformatics tools. The UVSSA protein performs a role in transcription-coupled DNA repair, which happens following exposure to high doses of UV radiation. The co-occurrence of these domains and the specificity of the PDE domain for cAMP and not cGMP may provide insight into the signaling mechanisms underlying repair processes triggered by UV light.

## *additional info*

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**Short- and long-distance communication in plants**

**Poster 106**

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# ROS and HCN-dependent NO formation *in vitro* and *in vivo*

Hydrogen cyanide (HCN) and nitric oxide (NO) are present in the environment and in organisms (plants and animals). Their chemical reactivity allows them to act as gasotransmitters or toxins, depending on concentration. HCN and NO are liberated during dormancy removal of apple (*Malus domestica* Borkh.) seeds. Seed dormancy breakage is linked to enhanced ROS level. HCN reacts with ROS, resulting in the generation of other compounds of signalling significance. The aim of this work was to demonstrate NO formation from HCN in the presence of hydrogen peroxide ( $H_2O_2$ ), *in vitro* and in axes of apple embryos. Axes of apple embryos shortly (5 h) pre-treated with 70 mM HCN (released from acidified potassium cyanide) were used for *in vivo* experiments, while *in vitro* studies were performed on an aqueous solution of HCN (175–700  $\mu$ M) with  $Cu^{2+}$  (20  $\mu$ M) and  $H_2O_2$  (1 M). Our data indicate that HCN stimulates the formation of nitrite ions (NO source), both *in vivo* and *in vitro*. HCN treatment enhanced NO emission, and the generation of  $O_2^{\bullet-}$  in plant tissue. The addition of HCN to the solution of  $Cu^{2+}$  and  $H_2O_2$  lowered  $O_2^{\bullet-}$  and  $\bullet OH$  levels *in vitro*, pointing to the formation of various NO derivatives.

**Short- and long-distance communication in plants**

**Poster 107**

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# Does the substrate matter? Analysis of noncanonical cyclic nucleotide generators in plants

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Adenylate and guanylate cyclases (ACs and GCs) are enzymes that synthesize the signaling molecules 3',5'-cyclic adenosine or guanosine monophosphate (cAMP or cGMP) from nucleoside 5'-triphosphate ATP or GTP, respectively, while phosphodiesterases (PDEs) hydrolyzes them into their inactive monophosphate forms. Together, these enzymes tightly regulate the cellular levels of cyclic nucleotides. Although alternative cyclic nucleotide uridine 3',5'-cyclic monophosphate (cUMP) have long been hypothesized to exist in plants, only recent advances in high-resolution mass spectrometry have provided some evidences of its presence but the specific enzymes generating cUMP remain unidentified. Given that ACs and GCs often exhibit low substrate specificity, it has been proposed that they might also convert UTP into its cyclic form. In this study, we investigated whether known ACs and GCs can catalyze the conversion of pyrimidine nucleotide UTP into its corresponding cyclic form, cUMP. We also examined the capacity of established plant PDEs to hydrolyze this noncanonical cyclic nucleotide. Finally, we present a bioinformatic analysis aimed at identifying potential candidates for uridylate cyclases (UCs) in plant genomes, highlighting sequence motifs and domain architectures that may underlie this activity.

## *additional info*

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**Short- and long-distance  
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