PLANT
BIOLOGY
EUROPE 2025
BUDAPEST

PROGRAMME AND BOOK OF ABSTRACTS

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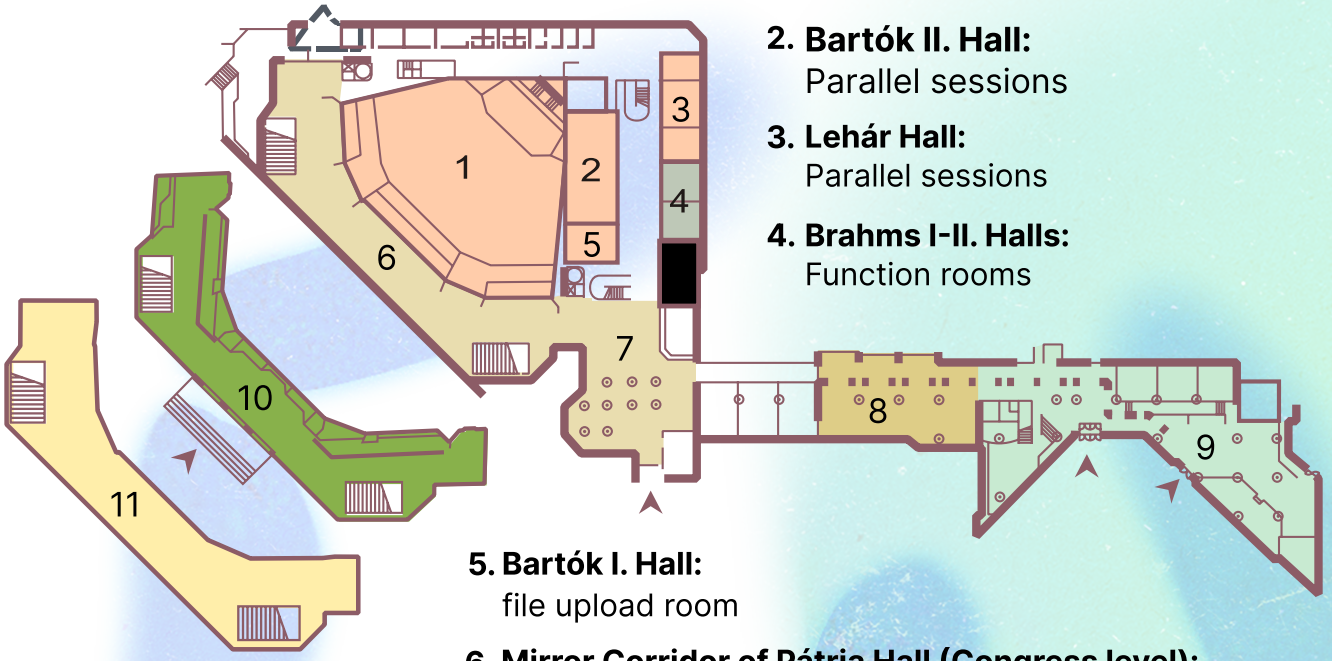
Legend



1. **Pátria Hall:**
   Plenary and parallel sessions
2. **Bartók II. Hall:**
   Parallel sessions
3. **Lehár Hall:**
   Parallel sessions
4. **Brahms I-II. Halls:**
   Function rooms
5. **Bartók I. Hall:**
   file upload room
6. **Mirror Corridor of Pátria Hall (Congress level):**
   exhibition, coffee break,
7. **Aula:**
   registration, coffee break, lunch, welcome reception
8. **Arcade Restaurant:**
   lunch
9. **Côté Jardin Restaurant:**
   lunch
10. **Entrance Corridor (Entrance level):**
    poster session
11. **Gallery Corridor (Gallery level):**
    poster session

PLANT BIOLOGY EUROPE 2025
BUDAPEST

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Detailed programme of the Congress days, including the social events

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List of accepted posters, ordered by poster ID grouped the final accepted session

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40 GENERAL INFO

Our sponsors

ELSEVIER

Molecular Plant

Frontiers in Plant Science

A journal by frontiers

Springer Plant Cell Reports

Our exhibitors

ANIMALAB DISCOVERY PARTNER®

Hansatech Instruments

HYBRIGENICS SERVICES

biotech innovative

Journal of Experimental Botany

MDPI

IChemIm s.r.o.

PHENOSPEX Smart Plant Analysis

plantDitech Accelerates Phenotyping

CLF Plant Climatics

Plant Flow Solutions

PHOTON SYSTEMS INSTRUMENTS

UNION BIOMETRICA

WALZ Photosynthesis Instruments

Zealquest AI

WEDNESDAY

25 JUNE 2025

Check in - Arrival and registration		9:00-
Opening ceremony Chair: János Györgyey, President of FESPB		11:00 - 11:35
PL-1 László Erdei From FESPP to FESPB and beyond	**Pátria hall**	11:15 - 11:30
K-1 Christine Foyer Oxidative signalling in plants	**Pátria hall**	11:35 - 12:10
Lunch	**Restaurant of the venue**	12:15 - 13:15


Pátria Hall

	Redox biology Christine Foyer, Gábor Kocsy **Pátria hall**	**Morphogenesis and reproduction** Thomas Dresselhaus, Attila Fehér **Bartók room**	**Beneficial plant-microbe interactions** Péter Kaló, Stephan Pollmann **Lehár room**
13:15 - 13:40	**O-121** Gábor Kocsy Redox- and light spectrum-dependent modulation of microRNAs and their targets during stress adaptation	**O-93** Zoltán Magyar RETINOBLASTOMA-RELATED has both canonical and non-canonical regulatory functions during thermomorphogenic responses in *Arabidopsis* seedlings	**O-41** Raffaella Balestrini Exploiting tailored microbial consortia to enhance sustainability and resilience of grapevine cultivation
13:40 - 13:55	**O-122** Tibor Janda Light and temperature-dependent shoot-root signalling pathways in cereals	**O-94** Diksha Bhola Pectin chemistry effects non-uniform cell growth during mesophyll morphogenesis	**O-42** Valeria Todeschini Growth and secondary metabolite modulation by soil beneficial microorganisms in *Artemisia annua* plants
13:55 - 14:10	**O-123** Filipa Sousa Improving the resilience of chestnut plants to climate change: The role of stress priming	**O-95** Elisa Maricchiolo ERAD-mediated maturation of the regulatory protein of plant meristematic cells CLAVATA 3 emerged during evolution from algae to higher plants	**O-43** Joana Belo Cork endophytic microbiome: Characterization and impact on plant development
14:10 - 14:25	**O-124** Michela Molinari Unraveling sulphur metabolism and miR395 dynamics in salt-tolerant and sensitive rice varieties	**O-96** Pavel Pashkovskiy The role of phytochromes A and B in the regulation of fruit metabolism and chloroplast ultrastructure in tomato leaves under different ratios of red and far-red light	**O-44** Lalie Leclercq Unveiling the hidden allies of industrial chicory: A metagenomic exploration of rhizospheric microbiota and their impact on productivity and plant health
14:25 - 15:00	Coffee break		

session supported by: Molecular Plant

JUNE 25 26 27 28

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K-2 — **Pátria hall** — 15:00 - 15:40

Jiří Friml

Auxin signaling revisited: New roles for second messengers

PL-2 — **Pátria hall** — 15:40 - 16:10

FESPB Award talk - Noel Blanco-Touriñán

How to build a root system:
A focus on hormone perception, specificity, and vascular connections

Abiotic stress - Osmotic Jolán Csiszár, Tibor Janda **Pátria hall**	**Morphogenesis and reproduction** Thomas Dresselhaus, Attila Fehér **Bartók room**	**Beneficial plant-microbe interactions** Péter Kaló, Stephan Pollmann **Lehár room**	16:15 - 17:25
O-05 Mai-He Li Root carbon shortage leads to mortality of drought-stressed trees	**O-97** Martin Bayer How parental factors shape the plant embryo	**O-45** Stephan Pollmann *Serendipita indica* promotes *Arabidopsis thaliana* growth through nuanced modulation of auxin homeostasis and transport	16:15 - 16:40
O-06 Bekim Gashi Recovery of photosynthetic activity in resurrection plants *Ramonda serbica* and *Ramonda nathaliae* after freezing-induced desiccation	**O-98** Dunja Leljak-Levanić Somatic embryogenesis is an ancestral reproduc-tive strategy in plants	**O-46** Bruno Sousa Enhancing tomato plants' resilience to climate change: The role of mycorrhization and strigolactones	16:40 - 16:55
O-07 Youcef Haddad Elucidate the reality and functionality of Natural Deep Eutectic Solvents (NaDES) formation in desiccation tolerant seeds	**O-99** Davide Gerna Intracellular glass fragility distinguishes the desiccation response of embryonic axes from recalcitrant and orthodox seeds	**O-47** Rui Lima Symbiotic NCR-Like peptides and bacteroid differentiation in *Amorpha fruticosa – Mesorhizobium amorphae* symbiosis	16:55 - 17:10
O-08 Laura Zsigmond Mitochondrial electron transport mutants modulate drought tolerance in arabidopsis	**O-100** Afsheen Malik Insights into the sex determination mechanisms and inheritance of dioecy and monoecy traits in *Cannabis sativa* (hemp)	**O-48** Henrike Würsig Responses of maize roots and the rhizosphere microbiome to alternating precipitation: Insights from a three-year field study	17:10 - 17:25

session supported by: Molecular Plant

18:00 - 20:00

Welcome Reception

Location: Aula and mirror corridors

Celebrate the beginning of the congress, re-connect with old friends, meet new friends and network with industry partners at our welcome reception. Include snack and finger food buffet.

Included in all registration fees.

JUNE

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THURSDAY

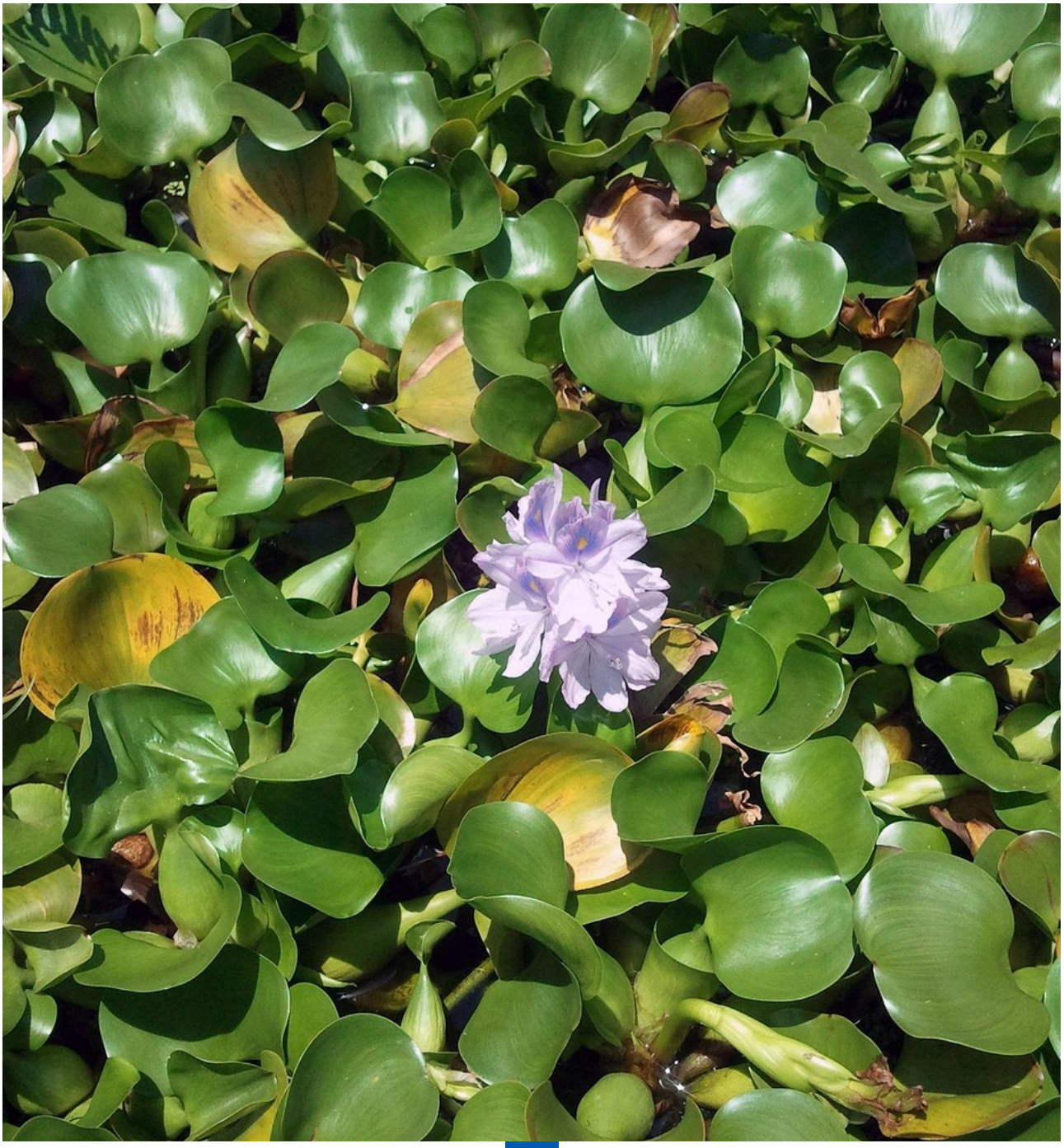
26 JUNE 2025

Registration desk open — 8:30-

K-3 — **Pátria hall** — 9:00 - 9:40

Marcel Jansen

Duckweed (*Lemnaceae*); The nutrient recyclers of the circular economy



JUNE

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9:45 - 11:00	**Abiotic Stress - Osmotic 2** Jolán Csiszár, Tibor Janda **Pátria hall**	**Aquatic plant biology and algal biotechnology** Marcel Jansen, Gergely Maróti **Bartók room**	**Nanomaterials in agriculture** Vasileios Fotopoulos, Ferenc Fodor **Lehár room**
9:45 - 10:10	**O-09** Ilona Mészáros Responses of intrinsic water-use efficiency and tree growth of sessile oak to climate change in North Hungarian central range	**O-33** Stefano Cazzaniga Turning a green alga red: Astaxanthin bio-synthetic pathway in *Chlamydomonas reinhardtii* improves resistance to light stress and biomass productivity at high irradiances	**O-101** Biljana Balen Interactive effects of silver nanoparticles and microplastics on oxidative stress in *Allium cepa* roots
10:10 - 10:25	**O-10** Guido Domingo The root code cracked: Deciphering wheat exudation during drought	**O-34** Rodrigo Bedera-García Inositol polyphospha-tes regulate resilient mechanisms in the green alga *Chlamy-domonas reinhardtii* to adapt to extreme nutrient conditions	**O-102** Pedro Diaz-Vivancos Effect of silver nanoparticles (AgNPs) in apricot seedlings submitted to mild salinity
10:25 - 10:40	**O-11** Magdalena Korek Mutation in a barley strigolactone repressor *HvD53A* impairs photosynthesis but increases drought tolerance	**O-35** Matteo Ballottari Abiotic stress-induced chloroplast and cytosolic Ca^{2+} dynamics in the green alga *Chlamydomonas reinhardtii*	**O-103** Zoltán Molnár Integration of chitosan nanoparticles and cyanobacteria biomass in agricultural applications to enhance cereal crop productivity
10:40 - 10:55	**O-12** Davide Giordano Thermotolerance of natural *Arabidopsis* accessions from Catalonia growing under salinity	**O-36** Viktor Oláh A light touch: Protein output of duckweed cultures as a function of light intensity	**O-104** Aradhana Mishra Enhanced cellular internalization: A pre-dominant bactericidal mechanism of biogenic nanoparticles over chemical counterparts
11:00 - 11:30	Coffee break		

Abiotic Stress - Toxic metals Csaba Máthé, Ferenc Fodor **Pátria hall**	Aquatic plant biology and algal biotechnology Marcel Jansen, Gergely Maróti **Bartók room**	Immunity and plant-plant interactions Isabel Díaz, Lóránt Király **Lehár room**	11:30 - 12:40
O-21 Ferenc Fodor Heavy metal accumulation and tolerance in Szarvasi-1 energy grass	**O-37** Benedetta Mattei Modeling of microalgal growth and biochemical composition under nitrogen fluctuations	**O-85** Jakub Michalski Promising candidates for new PAMPs - how plants handle bacterial cyclic dinucleotides	11:30 - 11:55
O-22 Magdalena Krzesłowska Pectinous cell wall thickenings formation - a widespread defence strategy in plants growing on substrates polluted with toxic trace metals and metalloids	**O-38** Gergely Maróti Inter-kingdom interactions within natural and synthetic algal-bacterial communities	**O-86** Ravinder Goyal Crop root secretions hold promise of *Aphanomyces* root rot management in pulses	11:55 - 12:10
O-23 Francesca Lanni Evaluation of the sensitivity to inorganic arsenic of Carnaroli rice plant (*Oryza sativa* L.)	**O-39** Laura Morello Interspecific hybridis and polyploids in duckweed	**O-87** Hana Leontovyčov Pathogen's playbook: Phytohormone production and manipulation by *Leptosphaeria maculans*	12:10 - 12:25
O-24 Altafur Rahman Modulation of cadmium stress responses in wheat by putrescine pre-treatment under blue and white light		**O-88** Magdalena Arasimowicz-Jelonek Nitric oxide/nitroxyl interplay during plant-pathogen interaction	12:25 - 12:40
Lunch			12:40 - 13:45

13:45 - 14:25

K-4 **Pátria hall**

Éva Hideg

Antioxidant aspects of UV effects on leaves

14:25 - 14:55

PL-3 **Pátria hall**

FESPB Award talk 2 - Marta Nunes da Silva

Inside the host:
Mechanisms of tolerance and control against vascular pathogens

14:55 - 15:25

Coffee break

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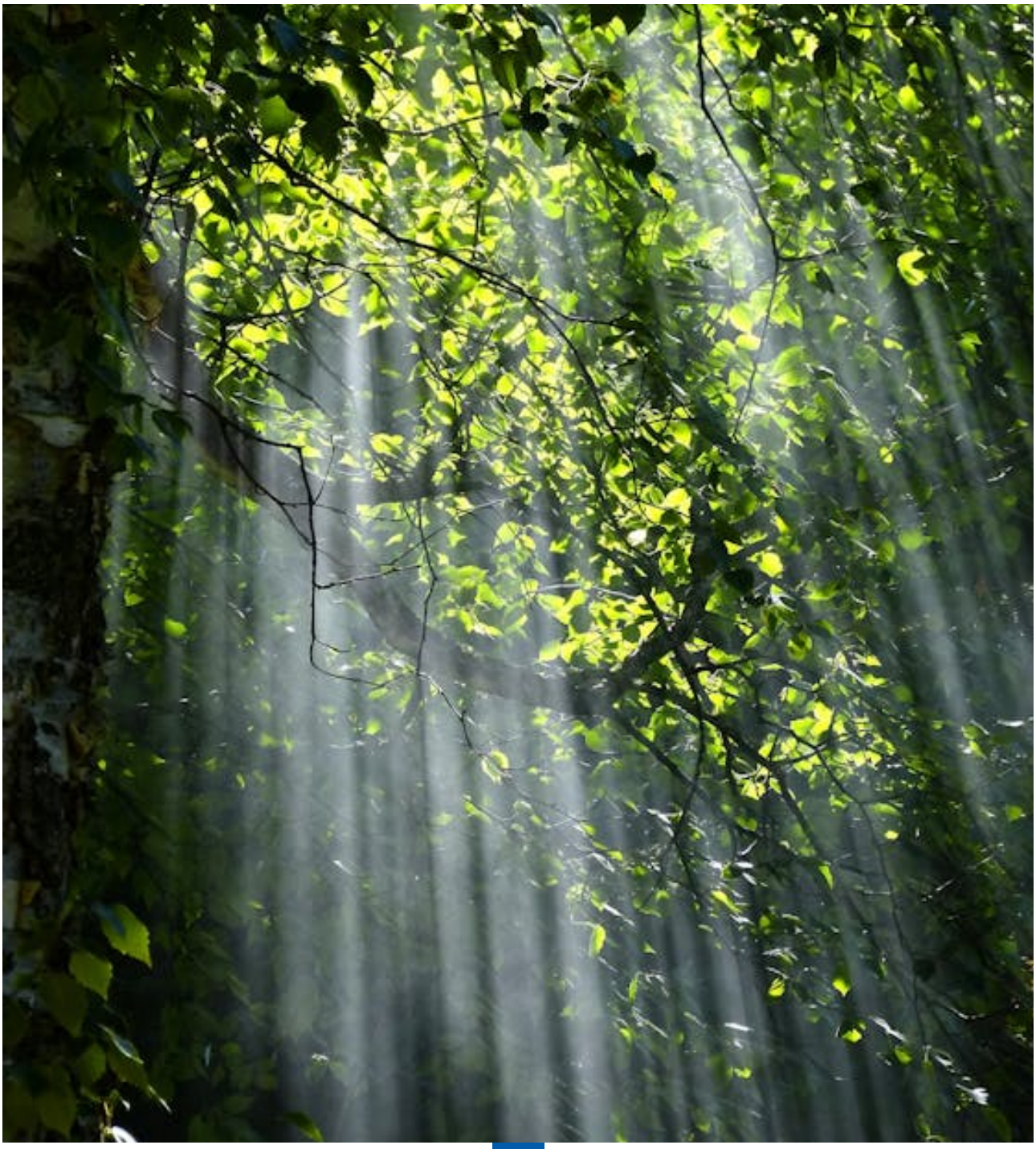

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Abiotic Stress - Temperature Laura De Gara, Tibor Janda **Pátria hall**	**Secondary metabolism** Éva Hideg, Gabriella Szalai **Bartók room**	**Immunity and plant-plant interactions** Isabel Díaz, Lóránt Király **Lehár room**	15:25 - 16:40
O-17 Sylva Prerostova High light as a tool for improving thermotolerance of rice plants through elevated cytokinin levels	**O-125** Antoine Mallavergne All in one: A serine carboxypeptidase-like protein catalyzes synthesis of chicoric and isochlorogenic acids in *Cichorium intybus*	**O-89** Yehoram Leshem Extracellular microRNAs: Plants' first line of defense?	15:25 - 15:50
O-18 Tibor Csorba Transcription fidelity contributes to heat stress survival in *Arabidopsis*	**O-126** Gaëtan Kientz Elucidation of the biosynthetic pathway of two C-glucosyl flavones accumulated in winter flax (*Linum usitatissimum* L.)	**O-90** Károly Fátyol Dissection of the antiviral RNA silencing pathway of *Nicotiana benthamiana* by genome editing	15:50 - 16:05
O-19 Luisa Friulla Heat tolerance and functional traits of Mediterranean tree species: A comparison between deciduous and evergreen species	**O-127** Camilo López-Cristoffanini Effect of a pre-harvest treatment with harvistatm on the nutraceutical quality and volatile compound profile of apples	**O-91** Éva Várallyay Investigation of VSR coding capacity of fruit tree infecting viruses	16:05 - 16:20
O-20 Magdalena Rys Physiological and biochemical changes occurring during deacclimation of oilseed rape – relation to frost tolerance	**O-128** Nadeera Yumali Wiyana Hewage Genetics and biochemistry of sticky trichomes	**O-92** Mesfin Haile Kebede Genotypic screening of *Capsicum* germplasm for disease resistance using fluidigm SNP markers	16:20 - 16:35
Posters	**Gallery and Entrance corridors**		16:40 - 18:10

FRIDAY

27 JUNE 2025

8:30- Registration desk open

9:00 - 9:40 **K-5** **Pátria hall**

Jaideep Mathur

Rapid sub-cellular responses for stress mitigation in plants

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Abiotic Stress - Practical aspects János Györgyey, Jolán Csiszár **Pátria hall**	**Secondary metabolism** Éva Hideg, Gabriella Szalai **Bartók room**	**Cell biology** Jaideep Mathur, Csaba Máthé **Lehár room**	9:45 - 11:00
O-13 Gregorio Barba-Espín Devising strategies to incorporate halophytes into the farming systems	**O-129** Magda Pál Polyamines in the "spotlight"	**O-53** Eunsook Park Chloroplast-nucleus communication: Protein trafficking for plant immunity	9:45 - 10:10
O-14 Unnikannan Prabhullachandran Long-term high temperatures affect seed maturation and seed coat integrity in *Brassica napus*	**O-130** Ágnes Szepesi Polyamine metabolism in microgreen plants during salt stress	**O-54** Nancy Soni From integrity to division: How cell wall status controls the cell cycle in plants	10:10 - 10:25
O-15 Andrea Sabia Impact of GenX on the physiology of *Solanum lycopersicum*: Implications for agriculture and environmental safety	**O-131** Douaa Bekkai Cadmium stress in *Salvia* species: Unveiling the phenolic profile, antioxidant potential and toxicity assessment	**O-55** Dhika Amand The impact of cell shape on cellular microviscosity dynamics in response to abiotic stress-induced-cell wall integrity impairment	10:25 - 10:40
O-16 Shubhi Mishra Chronic ionizing radiation impact on pathogen resistance in aquatic plants	**O-132** José Manuel Martí-Guillén Biotechnological production of a yellow carrot cell culture enriched in phytosterols and phenolic compounds and evaluation of its potential biostimulant effect in tomato seeds	**O-56** Almudena Gómez Unravelling transcriptional changes at single cell resolution at early stages of Root Knot nematode infection	10:40 - 10:55
ELSEVIER	Coffee break		11:00 - 11:30

	Abiotic Stress - Molecular aspects Laura De Gara, Ferenc Fodor **Pátria hall**	**Application of genetic improvements** Zoltán Havelda, László Szabados **Bartók room**	**Cell biology** Jaideep Mathur, Csaba Máthé **Lehár room**
11:30 - 11:55	**O-1** Michela Zottini Role of mitochondrial unfolded protein response (UPRmt) in abiotic stress tolerance	**O-25** Amedeo Moine Exploiting somaclonal variability to increase drought stress tolerance in grapevine	**O-57** Csaba Mathé The roles of PP2A subunits Fass and C3/C4 in the regulation of mitosis and oxidative stress responses in *Arabidopsis*- studies with phosphatase mutants and inhibitors
11:55 - 12:10	**O-2** Vasilissa Manova Plant growth regulators 4PU-30 and MEIA as promising modulators of antioxidant defense response in two wheat species exposed to drought and UV-B radiation	**O-26** Gagnon Penassou Genetic and physio-logical control of early vigor and transpiration efficiency in rice	**O-58** Eleonora Davidec AMP - A Tale of two messengers
12:10 - 12:25	**O-3** Paolo Korwin Krukowski Evolutionary insights into β-Cyclocitral signaling in *Physcomitrium patens*	**O-27** Yawar Habib Using molecular markers to combine freezing tolerance and grain quality in a durum wheat breeding program	**O-59** Edmund Kozieł Homogalacturonan pectins as an element of rbohD/F and rbohD response to TuMV infection
12:25 - 12:40	**O-4** Hilke Wittocx The role of FLA7 (*Bradi3g39740*) in salt stress response and plant development in *Brachypodium distachyon*	**O-28** Ewa Sobieszczuk-Noviczka Landscape and regula-tion of lncRNAs in pre-mature and develop-mental leaf senescence: A transcriptomic analysis in barley	**O-60** Omar Pantoja The chloroplast located HKT transporter plays an important role in fertilization and development in *Physcomitrium patens*
12:45 - 13:45	Lunch		

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K-6 **Pátria hall** 13:45 - 14:25

Thomas Dresselhaus

Peptides in the spotlight: Key players in plant reproductive process

14:30 - 15:45

Phytohormones and other transmitters Jiri Friml, Péter Poór **Pátria hall**	**Application of genetic improvements** Zoltán Havelda, László Szabados **Bartók room**	**High throughput phenotyping and remote sensing** Éva Darkó, Jolán Csiszár **Lehár room**
O-117 14:30 - 14:55 **Jitka Siroka** Salicylate profiling in plants in response to bacterial infection	**O-29** **Rachna Gowlikar** Building the edit suite: Foundational steps in hemp CRISPR technology	**O-81** **Éva Darkó** Utilisation of high-throughput phenotyping platform in Martonvásár
O-118 14:55 - 15:10 **Chwan-Yang Hong** 3-in-1 *in vivo* reporter system: Simultaneous detection of Auxin, GA and ABA phytohormonal responses and crosstalk in rice	**O-30** **Mohamed Kouhen** Validation of the DRO1 gene in tomato under drought stress: Insights into root architecture and drought adaptation	**O-82** **Daniel Provazník** From pixels to phenotypes: semi-automated classification of autumn reddening and bud set using hyperspectral imaging in scots pine
O-119 15:10 - 15:25 **Demetrio Marciano** A comparative analysis of receptor like kinases in Chlorophyta reveals the presence of putative cell wall integrity sensors	**O-31** **Alex Frimpong** Identification of Quantitative Trait Loci (QTL) underlying protein content in chickpea (*Cicer arietinum* L.) seeds	**O-83** **Davide Lucien Patono** Unraveling the mechanism of action of a biostimulant: a successful integration of eco-physiologycal investigation and high-throughput phenotyping
O-120 15:25 - 15:40 **Peter Poór** The light in the night: Role of reactive oxygen species and defence-related phytohormones in the action of nocturnal red light	**O-32** **Zoltán Havelda** Utilization of targeted mutations to alter economically important traits in barley	**O-84** **Ivan Kashkan** Sunlit insights: transferring hyperspectral imaging from lab to field

Coffee break 15:45 - 16:15

16:15 - 16:45

Dialogue with society - round table discussion
János Györgyey
Bartók room

PL-4
Mathieu Hanemian
Citizen science in action: developing vegetable intercropping and exploring plant-plant interactions

PL-5
András Badacsonyi
ERC for Plant Sciences

PL-6
János Györgyey
GMO-debate as an early example of modern science denial and spreading of pseudoscience in the post-truth world

16:45 - 18:15 Posters **Gallery and Entrance corridors**

18:30 - Departure to the Gala Dinner

18:30 - 23:00

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Folklore dinner with horseshow

Guests are welcomed with a horse show evoking the traditions of Hungarian equestrian sports, forest horse-drawn carriage rides, pony rides, animal petting and delicious Hungarian cuisine.

The 9-hectare park and the three different taverns of the training champion Lázár brothers provide an excellent setting for an unforgettable evening.

Not included in the registration fee!
Price: 90 EUR / person

Dress code is casual. The dinner place is outside Budapest. Bus transfer will be provided to the place of the programme and back to the venue. The bus ride takes about 50-60 minutes/direction.

SATURDAY

28 JUNE 2025

8:30- Registration desk open

9:00 - 9:40 **K-7** **Pátria hall**

Nils Stein

The barley pangenome

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Crop adaptation to climate change - Salinity Christian Zörb, Gyula Czégény **Pátria hall**	Biotechnology and synthetic biology Jolán Csiszár, László Szabados **Bartók room**	Nutritional homeostasis and water relations Ádám Solti, Tibor Janda **Lehár room**	09:45 - 11:00
O-69 Elide Formentin Regulation of root meristem size in rice by cytokinins and ROS under salt stress	**O-49** Pasquale Creanza Phaseolin, a new eco-friendly protein-based bioplastic	**O-105** Ádám Solti The impact of nitric oxide signal on the intracellular iron distribution	09:45 - 10:10
O-70 Sara Cimini miRNA-based regulatory networks: a key to enhancing salt tolerance in rice	**O-50** Ghulam Hasnain Metabolite repair in plants: A Nudix enzyme's role in thiamine salvage	**O-106** Oskar Siemianowski How do plants manage their microelements? Zinc translocation from Zn-sufficient to Zn-deficient roots as an adaptation to heterogeneous Zn availability	10:10 - 10:25
O-71 Sofia Spormann Combined and isolated effects of water deficit and salinity on *S. habro-chaites* and *S. galapagense*: A physiological approach	**O-51** Monika Danchenko Carnivorous sundew is a rich source of hydrolyses: A story of a peculiar protease	**O-107** Princia Nakombo Genetic control of the leaf ionome in pearl millet and correlation with root and agromorphological traits	10:25 - 10:40
O-72 Sara Esperanza Martínez-Lorente Biostimulants derived from plant cell cultures used as an alternative for ameliorating abiotic stress tolerance in Brassica seeds	**O-52** Raghuram Nandula G-protein alpha subunit negatively regulates nitrogen use efficiency in rice	**O-108** Brigitta Lantos Regulation effect of PH domain containing proteins on Mn transport and distribution	10:40 - 10:55
ELSEVIER	Coffee break		11:00 - 11:30

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11:30 - 12:45	**Crop adaptation to climate change - Drought stress** Christian Zörb, Gyula Czégény **Pátria hall**	**Photosynthesis and light regulation** Szilvia Zita Tóth, Péter Poór **Bartók room**	**Genomics and epigenomic** Nils Stein, Tibor Csorba **Lehár room**
11:30 - 11:55	**O-65** Dominik Großkinsky Old but gold: Exploiting the underutilized oilseed *Camelina sativa* to uncover and promote tolerance to abiotic stress for improving climate resilience in crops	**O-109** Luca Dall'osto Mapping light-harvesting function, photoprotection responses, and thylakoid stacking efficiency in the Photosystem II antenna system of plants	**O-73** László Szabados PEG-triggered osmotic stress generates large-scale transcriptional and epigenomic changes in rapeseed (*Brassica napus* L.)
11:55 - 12:10	**O-66** Alice Peduzzi Brassinosteroid seed-priming enhances the photosynthetic efficiency of *Sorghum bicolor* salt-sensitive hybrid	**O-110** Małgorzata Adamiec The role of *Egy2* protease in the regulation of chloroplast gene expression	**O-74** Ágnes Dalmadi A novel regulatory step of the miRNA pathway
12:10 - 12:25	**O-67** Patrick Lehr Guard cell metabolism – a key for regulating drought resilience?	**O-111** Supreeta Vijayakumar Optimising photo-synthetic efficiency *in silico* through species-specific Rubisco catalysis and improved protein allocation	**O-75** Payel Bhattacharjee Physiology shaped by environmental cues: Deciphering annual growth-dormancy cycle in Norway spruce (*Picea abies*)
12:25 - 12:40	**O-68** Chiara Pagliarani Effect of long-term stress memory on the regulation of grapevine responses to repeated drought events	**O-112** Miron Gieniec Beyond the leaves: unravelling the role of chloroplasts in the plant's stem	**O-76** Gaj Kerestes Pros and cons of targeted and high-throughput *BPM2* mRNA isoforms detection in *Arabidopsis thaliana*
12:45 - 13:45	Lunch		

K-8 **Pátria hall** 13:45 - 14:25

Ülo Niinemets

Plant stress in future climates: Responses and acclimation

14:30 - 15:45

Crop adaptation to climate change - Applications Christian Zörb, Gyula Czégény **Pátria hall**	**Photosynthesis and light regulation** Szilvia Zita Tóth, Péter Poór **Bartók room**	**Genomics and epigenomics** Nils Stein, Tibor Csorba **Lehár room**
O-61 14:30 - 14:55 **Chiara Pucciariello** Diversity of barley genetic resources for prompt seed germination after flooding events	**O-113** **Csaba Éva** Root-based inorganic carbon uptake boosts photosynthetic activity and osmotic stress tolerance	**O-77** **Petra P. Schrumpfova** Golem: A computational tool for exploring gene regulatory elements across the plant tree of life
O-62 14:55 - 15:10 **Clara Mata Martínez** Impact of climate change on peach fruit development and postharvest quality: insights from the WarmPeach project	**O-114** **Chiara Toffanin** Key factors enhancing growth by integrating green and far-red light in led lamps	**O-78** **Milton Gordillo** Genomic variation in the Andean lupin (*Lupinus mutabilis*): genome annotations, structural variations, and utility for breeding
O-63 15:10 - 15:25 **Serena Bordignon** Plants under stress: uncovering the role of miRNAs in rice adaptation to ionizing radiation	**O-115** **Yanli Zhang** Sugar infusion in trees: possibility, effects, and applications	**O-79** **Antonio Santiago Pajuelo** On the building of PlantaeViz, an integrated omics platform for non-model and crop plant species
O-64 15:25 - 15:40 **Maria Teresa Chiofalo** From sea to soil: *Saccharina latissima* as a natural soil amendment	**O-116** **Aida Shomali** Regulation of photosynthesis in farred-insensitive mutants of tomatoes	**O-80** **Sookyeong Lee** Genome-Wide association study reveals novel QTLs associated with lignan content in sesame accessions from the RDA-Genebank

Closing Ceremony 15:45 - 16:30

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PRESENTERS' INFO

POSTER PRESENTATIONS

Poster format: A0, portrait (841 x 1189 mm).

Pins and adhesive tapes will be provided to fix the posters.
Poster presenters of ALL poster numbers are able to
mount their posters from **10 AM 25 June 2025**,
(or latest till the beginning of the first poster session)
and remove them on Saturday, **28 June till 13:30 the latest**.

Posters will be identified by poster numbers, which are indicated in the programme booklet and in the conference application. Authors of posters should stand at their posters and be available to discuss their research during the Poster Sessions on Thursday or Friday according to the schedule in the final program.

POSTER SESSIONS

ODD numbered posters should be presented
on **Thursday, 26 June; 16:40 - 18:10.**

EVEN numbered posters should be presented
on **Friday, 27 June; 16:45 - 18:15.**

ORAL PRESENTATIONS

The schedule of the oral presentations can be seen in the detailed programme of this booklet. Speakers and session chairs are kindly requested to keep the time of the presentations. Make sure to bring your presentation file written on a USB flash drive. Presenters are kindly requested to give their presentation file to the technicians in the File Upload Room (Bartók I. room) preferably half day before beginning of the corresponding session.

Please note that speakers will not be able to use their own laptops!

LIST OF POSTERS

Application of genetic improvements

P-001 Namira Arfa
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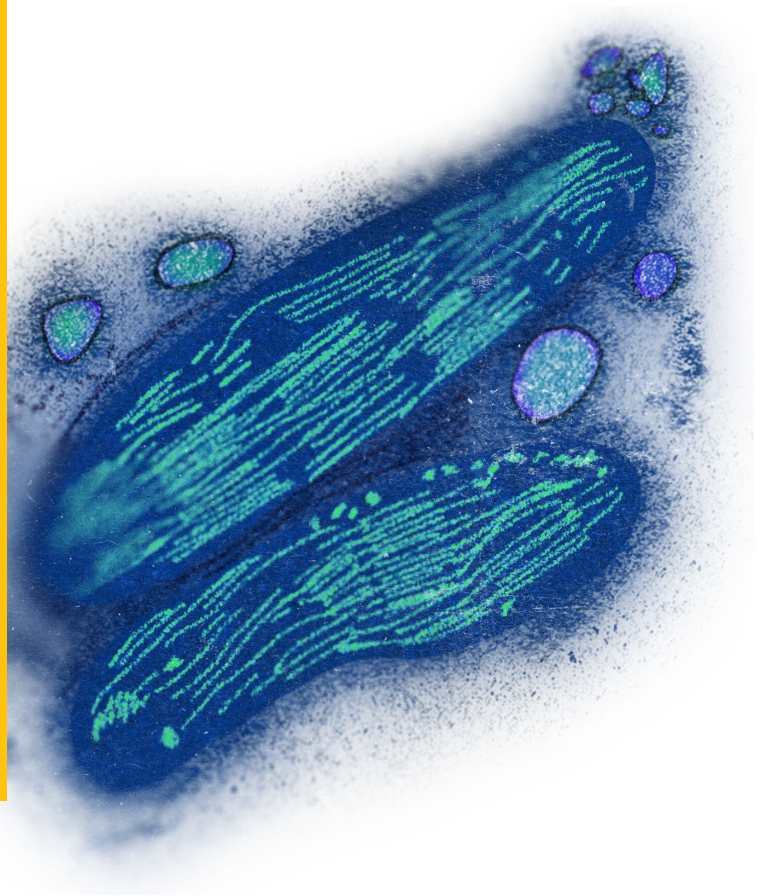

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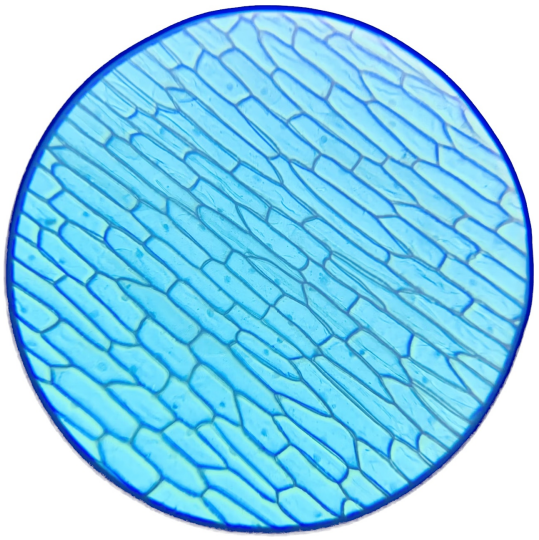

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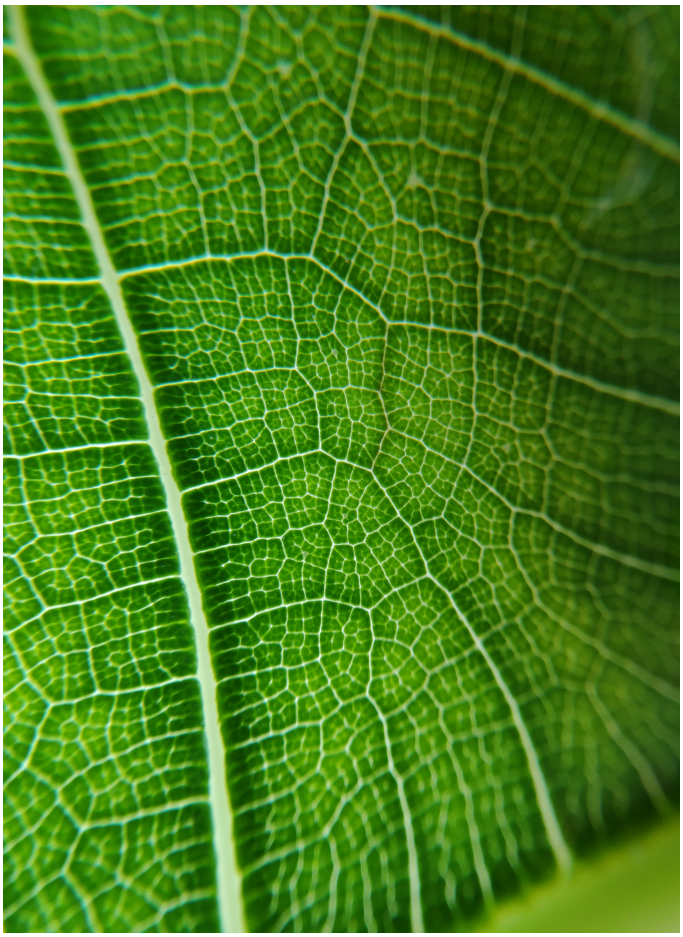

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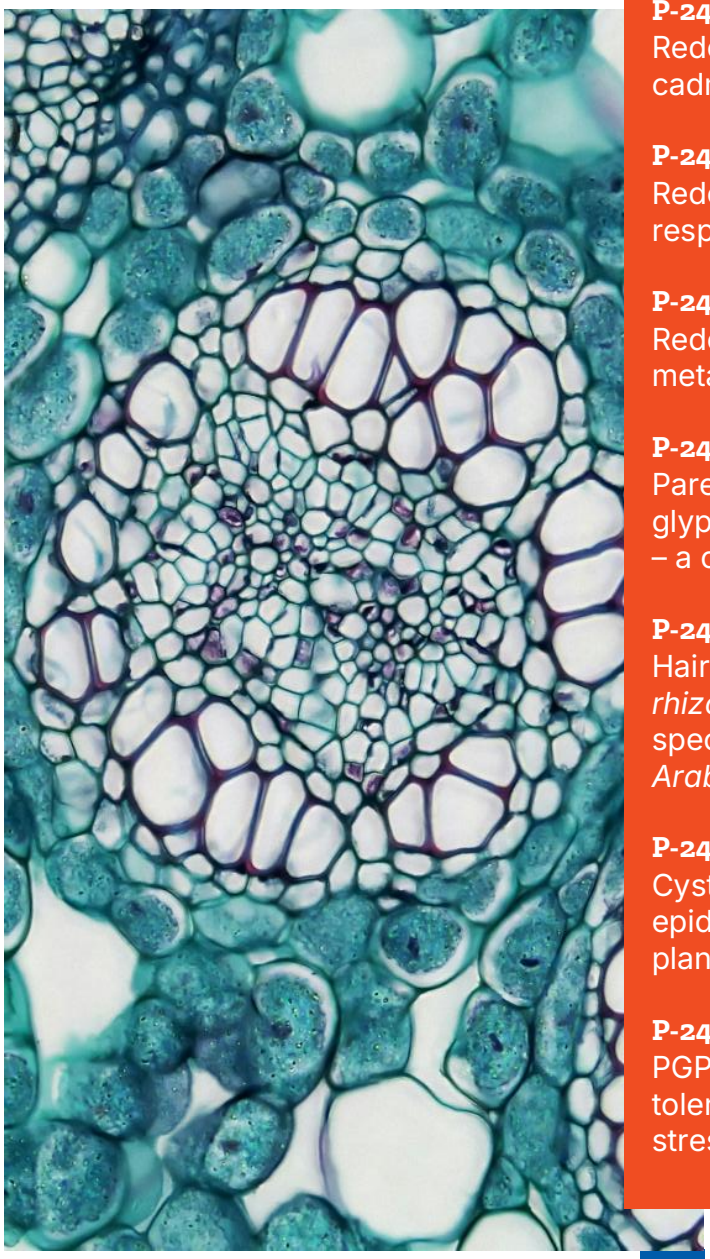

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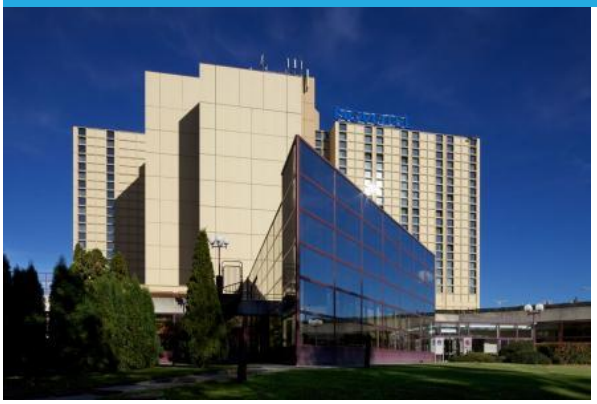

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PLANT BIOLOGY EUROPE 2025

BUDAPEST

KEYNOTE TALKS

K-1

Oxidative signalling in plants

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Keywords: hydrogen peroxide, redox proteome, catalase

Reactive oxygen species (ROS) are important signalling molecules in plants that play a crucial role in plant growth, development, and stress responses. ROS production and processing are tightly regulated to regulate cellular redox homeostasis and facilitate oxidative signalling. This presentation will discuss the considerable progress that has been made in our understanding of ROS sensing and signalling pathways in recent decades, while highlighting the significant gaps that remain in our current knowledge. I will consider oxidation of the nucleus in response to environmental stresses and consider the potential mechanisms of ROS processing in the nucleus. I will particularly focus on catalase as an enzyme that can be relocated to different cellular compartments. I will also consider re-programming of the redox proteome in response to changes in the levels of hydrogen peroxide generated in metabolism.

Finally, I will consider recent developments in systemic signalling, together with how they might be used to increase plant resilience to environmental stress.

■

K-2

Auxin signaling revisited: New roles for second messengers

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Keywords: Phytohormones, Auxin, ubiquitination, cAMP, cGMP

The plant hormone auxin is a versatile endogenous signal influencing virtually all aspects of plant life. It has a unique ability to be directionally transported through tissues, forming local auxin maxima and gradients that are central to many developmental processes mediated by auxin. One of the key roles of auxin is the adaptation of plant growth to gravity, where shoots bend up and roots bend down. This paradox is based on the opposite responses of these organs to the phytohormone auxin, which promotes cell expansion in shoots through a canonical mechanism while rapidly inhibiting it in roots via a yet unknown, non-transcriptional downstream mechanism.

Nuclear, transcriptional auxin signalling involves the TIR1/AFB auxin receptors, Aux/IAA transcriptional repressors, and ARF transcription factors. TIR1/AFBs are part of the ubiquitin ligase complex, mediating the ubiquitination and degradation of Aux/IAAs and thereby releasing ARFs from their inhibition. The unexpected identification of adenylate cyclase enzymatic activity in TIR1/AFB receptors (Qi et al., 2022) and the crucial importance of its product, cAMP, for the downstream regulation of transcription (Chen et al., 2025) revise this canonical model, which has withstood the test of time for 20 years.

Nonetheless, auxin also triggers cellular responses within seconds–too fast to rely on transcription. The classical rapid auxin responses leading to root growth inhibition are mediated by the non-transcriptional branch of TIR1/AFB signalling, mainly through AFB1. The downstream mechanism is still unclear but may involve guanylate cyclase activity of TIR1/AFBs targeting calcium channels.

I will present these and other mechanistic updates on transcriptional and non-transcriptional auxin signalling and show how these insights may serve as a blueprint for gaining a new understanding of other signalling pathways in plants.

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■

K-3

Duckweed (*Lemnaceae*): The nutrient recyclers of the circular economy

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Keywords: Lemnaceae, circular economy, plant nutrients

The concept of the circular economy dates back to the late 1960s but has in the last few years been adopted as a central tenet in European sustainability policies. The circular economy sets out to limit use of raw resources

through redesigning, reusing, repairing and recycling of products. It also sets out to reduce waste generation by treating waste as a new resource that can be valorised. In the case of agricultural and food processing industries, wastewater typically contains substantial concentrations of valuable plant nutrients. By retaining and valorising these plant nutrients eutrophication of local waters can be avoided, while an alternative to unsustainable use of synthetic fertilisers is created.

Duckweeds (*Lemnaceae*) are free floating aquatic plants that are widespread in slow flowing rivers, streams and freshwater lakes. Rapid growth, with in some cases doubling times from as little as two days, is paralleled by rapid uptake of plant nutrients from the water column. Thus, duckweed species can effectively capture and retain nutrients present in wastewater, and as a consequence remediate such waters. Valorisation relates to the potential replacement value of the resulting biomass as an alternative to imported soybean for use in animal feeds, based on its high protein content and good amino acid profile.

A substantial number of published studies has demonstrated growth of duckweed on a variety of different wastewaters, mostly under laboratory conditions. In the presentation, data will be presented on the growth of duckweed on meat-processing and dairy processing wastewaters, highlighting good growth as well as phytoremediation. However, a major challenge for the duckweed community remains scaling up of duckweed-based wastewater valorisation. Data will be presented on the development of successful indoor stacked duckweed bioreactors, as well as of one of Europe's largest outdoor duckweed growth systems, an integrated multitrophic farm in Co. Offaly, Ireland, where one hectare of duckweed takes up and retains nutrients released by some 30 tonnes of freshwater fish. The story of the progression from laboratory-based, small-scale experiments to semi-commercial circular economy applications will be concluded by an assessment of the steps required to progress from fundamental plant biological questions to commercial duckweed applications.

This work was supported by the Department of Agriculture, Food and the Marine (Ireland) under Grant Award No. 2021R487, and the European Union's Horizon Europe research and innovation programme under grant agreement No 101084437 – IMPRESS.

K4

Antioxidant aspects of UV effects on leaves

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Keywords: UV radiation, antioxidant, ROS

Solar ultraviolet (UV, 280-400 nm) radiation is a specific regulator of gene expression, metabolite biosynthesis, and responses to climate change parameters. However, UV, especially the high-energy UV-B (280-315 nm) range, can also act as a stress factor if it occurs together with other adverse environmental effects (Jansen et al. 2019). Even when plants are not suffering from damaging levels of stress, physiological and metabolic responses to UV radiation depend not only on the intensity and spectral range of UV, but also on the ratio of PAR to UV. Acclimative physiological and metabolic responses to various UV ranges include changes in the regulation of photosynthesis, as well as increased antioxidant enzyme activities and non-enzymatic antioxidant capacities. The latter serve as regulators of ROS levels in UV exposed leaves, which, in addition to photoreceptors (Jenkins et al. 2001), are assumed to prompt to defence against UV (Hideg et al. 2013). Specialized metabolites, such as flavonoids and phenolic acids promote acclimation as UV absorbing molecules in addition to their antioxidant function. UV responses can be explored in outdoor experiments with plants acclimated to various UV ranges, and also in indoor studies using artificial UV sources. Experiments conducted in plant growth chambers provide models that isolate individual factors, and also contribute to finding indoor farming options that improve the nutrient values of plants. The presentation summarizes results of our experiments with tobacco and Arabidopsis model plants on the above topics, demonstrating, among other aspects, the importance of efficient H_2O_2 housekeeping in UV exposed leaves (Czégény et al. 2014, Czégény & Rácz 2023), the versatile role of phenolic compounds (Csepregi & Hideg 2018, Csepregi et al. 2025), and the occurrence and importance of systemic UV responses.

The authors are grateful to all international cooperating partners who shaped their perspectives during joint research projects or conference and workshop discussions within the framework of COST Action UV4Growth and the UV4Plants Association for Plant UV Research.

Work at the University of Pécs is currently supported by the National Research, Development, and Innovation Office (Grant numbers K-142419 and PD-142420).

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K5

Rapid sub-cellular responses for stress mitigation in plants

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Keywords: Stress, Subcellular interactions, Plastids, Fluorescent-proteins

Although plants are unable to move, they are amazingly resilient and can overcome a wide range of stresses. If left untended, stress, colloquially defined as "Something Requiring an Effective Solution Soon" [1], can have severe negative consequences for living cells. While a major preoccupation of living cells is to continuously adjust to the stress and maintain homeostasis for optimal functioning a perusal of literature on plants shows that our awareness of plant response is very often based on observations made long after the causal event has occurred. I hypothesized that plants possess a sub-cellular response machinery that quickly refashions existing cellular resources and thus minimize the negative effects of transient stress. This 'rapid' response machinery was expected to be different from the 'long-term' changes that occur in plants during acclimation and adaptation.

By 2005, a large number of organelle-targeted fluorescent fusion proteins had become available and became the tools for my investigations aimed at understanding the rapid subcellular responses machinery in plants. Drawing on pertinent observations, collected over three decades of research, my talk will draw attention to several transient subcellular phenomenon that occur rapidly in plants responding to stress. Very often, organelle shapes change quickly and include the transient formation of tubular extensions called peroxules, stromules and matrixules. The changes often reflect subtle alterations in the composition and flexibility of bounding organelle membranes [2] and are readily visible in plastid behaviour. I will provide strong evidence to show how plastid-derived lipids are able to reach other organelles quickly and increase the overall cellular resilience to stress. Interestingly, under optimal, non-stressed conditions of growth most organelles maintain their independent functions without physically interacting with each other. However, all organelles interact with the endoplasmic reticulum (ER) to form microdomains within the cell [3,4]. Based on my findings the concept of ER-microdomains interacting to formulate a singular cellular response to stress will be discussed.

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[2] Membrane contacts with the endoplasmic reticulum modulate plastid morphology and behaviour. 2023. doi: 10.3389/fpls.2023.1293906

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K6

Peptides in the spotlight: Key players in plant reproductive process

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Keywords: plant reproduction, peptide signaling, fertilization mechanisms, PCD

In contrast to animals and early land plants like mosses and ferns, sperm cells of flowering plants have lost their motility and are transported and protected as a passive cargo by pollen grains and pollen tubes, respectively. After (i) adhesion of pollen grains at papilla cells of the stigma, (ii) during their hydration, (iii) during pollen germination and (iv) further growth and guidance of pollen tubes towards the egg apparatus inside ovules, and finally (v) during sperm cell release, intensive crosstalk takes place between pollen grains/pollen tubes and the surrounding maternal and female gametophytic tissues. During recognition processes, pollen of the own

species is promoted, foreign pollen rejected, and mechanisms were established to avoid self-fertilization. Polymorphic peptides are at the center of these various communication processes. Among the various classes of peptides especially small-secreted cysteine-rich peptides (CRPs) are involved as mobile ligands in the diverse cell-cell communication events along the pollen tube journey.

By focusing on Arabidopsis and maize as eudicot and monocot model plants, respectively, I will report among others on the role of pollen-, papilla- and embryo sac-expressed CRPs of the RAPID ALKALINIZATION FACTOR (RALF) family and their interaction with malectin-like receptor kinases of the CrRLK1L family, GPI-anchored LORELEI-like proteins (LLGs) and leucin-rich repeat extensin-like (LRX/PEX) cell wall proteins. RALF signalling results in Ca^{2+} spiking, ROS production, and partly also in programmed cell death (PCD). The role of further polymorphic peptides will be discussed, their role in speciation mechanisms and applications to overcome hybridization barriers and to generate novel plant species.

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K7

The barley pangenome

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Keywords: barley, pangenome, genebank

The genomic revolution, initiated by the introduction of high-throughput next generation sequencing technology combined with new compute power and algorithms, has made de novo sequencing and assembly a routine task, almost independent of genome complexity. While sequencing of the multi-gigabase genomes of the Triticeae crop species barley, wheat, rye initially required the collaboration of larger research consortia, complete sequencing of Triticeae genomes is now a routine task in frame of a map-based cloning project. This opens new perspectives: crop genome diversity can be captured today at species' scale. Entire genebank collections of barley are / were genotyped by sequencing, providing global population diversity resolution. This is providing now foundational information for pangenome sequencing. In frame of an international effort, more than 75 barley genomes, including 23 wild *H. vulgare* ssp. *spontaneum*, were assembled into chromosome scale scaffolds using PACBIO Hifi and Hi-C data (1) substantiated by a pan-transcriptome of 20 of the cultivated genotypes (2). This pangenome is representative for most of the pericentric haplotypes in extant barley germplasm as well as in genetic resources, however, in order to capture rare allelic diversity and especially the highly recombinogenic telomeric ends of barley chromosomes, a continued effort of discovery by genome sequencing is required and has resulted so far in more than 100 additional draft or high-quality chromosome-scale assemblies. In addition, pangenome initiatives are working towards a genus-*Hordeum*-pangenome, providing access to the allelic diversity of barley's secondary and tertiary genepool. Altogether these resources provide a rich foundational source of information for biological research and future crop improvement.

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[2] Guo, W, Schreiber, M, Marosi, V, et al. (2025) A barley pan-transcriptome reveals layers of genotype-dependent transcriptional complexity. Nat Genet. 10.1038/s41588-024-02069-y

■

K8

Plant stress in future climates: Responses and acclimation

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Keywords: Global change, elevated CO2, interspecific variability, stress resistance

Globally change is expected to increase the frequency and severity of different abiotic and biotic stress episodes and lead to more frequent stress interactions. How plants cope with sequential and interacting stresses is poorly understood. Furthermore, the increase of ambient CO_2 concentration ($[CO_2]$) alters both plant primary and secondary metabolism and can importantly modify plant sensitivity to various stresses. In particular, elevated $[CO_2]$ is expected to improve plant heat and drought resistance, but the evidence of the impacts of elevated $[CO_2]$ on herbivory resistance is contrasting. Furthermore, the sensitivity and response magnitude to global change-driven increases in stress prevalence strongly vary among plant species and this is associated with interspecific variability in investments in constitutive and induced defenses and associated trade-offs. The current presentation first highlights the basic concepts of stress response, acclimation and resistance, and reviews the key phenotypic responses to drought,

heat and herbivory stresses. Then the presentation demonstrates how interactive stresses alter plant phenotype and ultimately discusses the direct and indirect impacts of elevated [CO_2] on stress response and acclimation. The presentation emphasizes the suites of chemical, physiological and structural plant traits that determine the interspecific variability in stress resistance and identifies the key knowledge gaps for future studies. The presentation concludes that the prediction of vegetation performance in future climates requires characterization of interspecific variation in stress responses and process-based understanding of how stress responses are affected by elevated [CO_2].

PLANT BIOLOGY EUROPE 2025

BUDAPEST

PLENARY TALKS

PL-1

From FESPP to FESPB and beyond

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In this retrospection the past five-decade long history of plant science congresses is shown from the birth of the Federation of European Societies of Plant Physiology (FESPP) and its renamed form of the Federation of European Societies of Plant Biology (FESPB) till present.

The interim meetings and the foundation of FESPP dates back to the mid 1970's and the Inaugural Congress was held in Edinburgh 1978. The change of the name from FESPP to FESPB took place in 2002 at the 13th Congress in Hersonnisos, Crete, counting the congresses continuously afterwards.

This year we remember the 25th anniversary of the 12th FESPP Congress held in Budapest, 21-25 2000, an outstanding an memorable occassion the bimillenary and also the Millenium of the Hungarian Christian statehood.

Finally the scientific topics of the former 12th and the present 24th Congresses and changes during a quarter of century are compared.

PL-2

How to build a root system: A focus on hormone perception specificity and vascular connections

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Life on Earth originated approximately 3.7 billion years ago, with land plants emerging around 450 million years ago from a lineage of freshwater algae. The transition from aquatic to terrestrial environments required significant morphological, physiological, and developmental adaptations. Key innovations during this transition included the development of roots and vascular tissues.

The architecture of the root system is crucial for proper plant development and is dynamically shaped in response to environmental stresses. Among the key regulators of root development are the phytohormones brassinosteroids. For almost two decades, it was believed that brassinosteroids could direct growth in a non-cell autonomous manner. However, our results challenged this view and demonstrated that brassinosteroid signaling largely operates in a cell-autonomous manner.

A well-organized root system depends not only on the proper formation of the primary root, but also on the establishment of a continuous vascular network. This network connects newly formed lateral roots–which contribute to developmental plasticity–to the main root. I will also discuss the molecular and cellular mechanisms that preserve vascular connectivity during post-embryonic growth.

PL-3

Inside the host: Mechanisms of tolerance and control against vascular pathogens

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Keywords: elicitors, integrated pest management, kiwifruit bacterial canker, phytopathogens, pine wilt disease, plant tolerance

Vascular pathogens represent a growing challenge for plant health, particularly in the context of global trade and climate change. Their ability to colonize and spread systemically through the xylem makes them especially destructive and difficult to manage. Once inside the vascular system, these pathogens often evade external defences and rapidly disrupt water and nutrient transport, leading to severe wilting, dieback, and plant death. Despite their impact, our understanding of the plant traits that determine susceptibility or resistance remains limited.

In this work, we explore two emblematic pathosystems, the pine wilt disease caused by the nematode *Bursaphelenchus xylophilus* (Bx) and the kiwifruit canker caused by the bacteria *Pseudomonas syringae* pv. *actinidiae* (Psa), to investigate the mechanisms underlying host tolerance and sustainable defence activation. In pine trees, *Pinus pinea* displayed tolerance to Bx through a combination of structural traits (e.g., fewer resin canals in the xylem and cortex, smaller tracheids) and biochemical defenses (e.g., higher constitutive antioxidant levels and rapid induction of flavonoids and phenolics). The exogenous application of defense elicitors, such as chitosan and methyl jasmonate, significantly reduced nematode proliferation and disease

severity by priming host defence responses, particularly antioxidant capacity, in susceptible species such as *P. pinaster*.

In kiwifruit, comparative transcriptomic and gene expression analyses between *Actinidia chinensis* (susceptible) and *A. arguta* (tolerant) revealed earlier activation of defense signaling and phenylpropanoid biosynthesis pathways in the tolerant genotype. Hormonal profiling showed a more balanced JA/SA response, with early transient peaks and more effective stomatal regulation in tolerant plants. Additionally, nitrogen supply in the form of ammonium appeared to impair plant tolerance, while certain essential oils demonstrated potential for Psa prevention or treatment depending on their composition.

Together, these findings highlight the value of integrating anatomical, biochemical, and molecular traits to inform sustainable control strategies against systemic pathogens. Such integrative approaches are increasingly urgent as climate change continues to expand pathogen ranges and intensify disease pressure.

This work was supported by national funds through FCT – Fundação para a Ciência e a Tecnologia (project references: 2023.06124.CEECIND and UID/Multi/50016/2020).

PL-4

Citizen science in action: Developing vegetable intercropping and exploring plant-plant interactions

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Keywords: agroecology, intercropping, citizen science

In the face of global change, the conventional agricultural model must evolve to reduce its environmental impact and be more resilient. In this context, agroecology offers a conceptual framework to respond to these issues by reintegrating natural regulations back at the heart of agricultural systems to allow farmers and civil society to benefit from their ecosystem services, and therefore improve global health. Increasing the yields of market garden crops and regulating bio-aggressors is thus possible thanks to plant diversification, such as vegetable intercropping, tested empirically in gardens and the professional sector, particularly in (peri-)Urban Agriculture, but for which scientific data are scarce. To sustainably develop vegetable intercropping, rigorous field studies are needed integrating the complexity of the socio-agronomic processes involved. In this interdisciplinary project, citizen-gardeners, market gardeners and scientists from various disciplines collaborate to explore vegetable intercropping in its many dimensions.

Together, we designed a participatory experiment in which tomatoes and beans are intercropped and tested by dozens of amateurs and professionals. Conducted in 2024 and 2025, this experiment records the yields of each crop, both as sole crops and in intercropping, to quantify the agronomic success of the system. We also examine the relationship between soil physico-chemical properties and microbiota composition to investigate the impact of intercropping, as well as ecosystem services (weed control, water regulation, ...). We integrate human sciences to understand the sociotechnical constraints to the development of vegetable intercropping through a quantitative survey as well interviews.

By fostering mutual knowledge between scientists and civil society, we want to improve the relevance of our experimental systems in line with society needs and increase the impact of our research. Through participation to the experiment, citizens also gain knowledge, learn the scientific method, and become active agents of information dissemination. The methods used to build the project with citizens and the first socio-agronomic results will be presented.

PL-5

ERC for plant sciences

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The European Research Council (ERC), set up by the European Commission in 2007, is the premier European funding organisation for excellent frontier research. The ERC's mission is to encourage the highest quality research in Europe through competitive funding and to support investigator-driven frontier research across all fields, based on scientific excellence.

Through its long-term grants, it provides scientific freedom and financial stability to its grantees and the reputation of excellent research. ERC projects have led to more than 200'000 articles published in scientific journals, over 2'200 patents and other IPR applications,

and ERC grantees founded or co-founded over 400 start-ups so far.

Based on my fourteen years of experience working at the ERCEA, I will attempt to demonstrate how ERC and its projects contributed to the progress of the various plant science through data and successful project examples. I will present the available funding options and the peer-review evaluation process, and I will give some tips for those who intend to apply for an ERC grant in the future.

PL-6

GMO-debate as an early example of modern science denial and spreading of pseudoscience in the post-truth world

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Keywords: GMO, science denial, failed dialogue

Over two decades of knowledge dissemination activities, I have observed that rational, argumentative, and educational presentations have become increasingly less effective. The field of plant gene technology is among the most striking examples of this phenomenon, as it has failed to achieve public acceptance of genetically modified (GM) plants in agriculture.

The term "GMO" lost its original meaning in public discussions. It has become a stigmatizing umbrella term for agro-biotech developments, encompassing economic, political, financial, anti-globalization, and even human rights issues in the minds of most European citizens. Since the introduction of the first GM crops into fields, the anti-GMO green movement has simplified the complex issues into a straightforward, superficial "black and white" question, successfully presenting the issue to the public as a struggle between good and evil.

In the meantime, the scientific community has attempted to provide the public with a nuanced and detailed education about gene technology, explaining the technology behind it, its clear advantages, and its potential usefulness. However, it was a nearly complete failure, we have to admit. The fear monger, based on pseudoscience, falsified research results, misbeliefs, or unrealistic assumptions, was much more effective. In a few years, we found ourselves on the wrong side, being labeled as "shills of the Big Agro" in this oversimplified post-truth world.

Thanks to the advancement of gene editing, plant gene technology has progressed to much more advanced levels. In research, gene editing has become an indispensable tool; high-end plant molecular biology work almost certainly involves it. However, the public perception is hardly changing, and the tedious process of learning scientific knowledge has become even less popular. We risk that the ongoing modernization of the regulation of plant gene technology (NGT/NBT) in the EU will spark similar emotion-based public resistance, as it did around the millennium, and we may find ourselves more or less in the same dead end.

Therefore, we must change our communication strategy and learn what I define as science-based storytelling: simple, easy to understand, yet still accurate, scientifically proven tales about individual developments, such as Golden Rice, *Phytophthora*-tolerant potato, or drought-tolerant wheat. We may need to involve citizens in the preliminary investigations, making them aware of new developments and progress towards a more sustainable agriculture.

PLANT BIOLOGY EUROPE 2025

BUDAPEST

ORAL PRESENTATIONS

O-1

Role of mitochondrial unfolded protein response (UPRmt) in abiotic stress tolerance

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Keywords: Unfolded Protein Response, mitochondria, abiotic stress

Mitochondria are key organelle involved in many different cellular processes, from the production of energy to stress sensing and response. However, to preserve proper organelle function, is necessary to maintain mitochondrial protein homeostasis. This is usually assured by the action of the mitochondrial protein quality control (mtPQC), a complex network of chaperones and proteases. However, when there is an accumulation of misfolded/unfolded proteins that goes beyond its capability the cell mounts the mitochondrial unfolded protein response (UPRmt).

In this work, some new candidate UPRmt marker genes were identified in Arabidopsis thaliana seedlings. These markers were then used to better understand the role of UPRmt in the response to abiotic stresses. The comprehensive data generated from this study provides compelling evidence that UPRmt exerts a significant influence on the plant's ability to respond to a range of environmental stressors, including but not limited to elevated temperatures, osmotic imbalances, and high salinity conditions. The findings suggest that UPRmt plays a crucial role in modulating the plants' overall stress tolerance and adaptability, potentially opening up new avenues for enhancing crop resilience in the face of climate change and other environmental challenges.

This work was supported by the grants PRIN_PNRR Prot. P2022B4C5H

O-2

Plant growth regulators 4PU-30 and MEIA as promising modulators of antioxidant defense response in two wheat species exposed to drought and UV-B radiation

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Keywords: oxidative stress, wheat, gene expression

Abiotic stresses are among the main causes of reduced crop growth and productivity. In recent years, alternative wheat species such as einkorn (*Triticum monococcum*, L.) have attracted increasing interest due to their inherent stress-tolerance and better processing characteristics and nutritional value of the grain, compared to modern wheat varieties. An early primary response of plants subjected to abiotic stress is a "burst" of reactive oxygen species (ROS). Plants have evolved enzymatic and non-enzymatic mechanisms to neutralize reactive oxygen species and maintain redox balance. A promising way to reduce the harmful effects of stress is treatment with non-toxic substances that activate various defense mechanisms in plants. This work has investigated the biochemical and molecular characteristics of the stress response of einkorn and wheat seedlings exposed to drought or UV-B radiation with a focus on the potential of exogenously applied plant growth regulators to activate plant defense mechanisms. For this purpose, control plants and those pretreated with 4-PU30 (phenylurea type cytokinin 4PU-30) or MEIA (β-monomethyl ester of itaconic acid) were subjected to the respective abiotic stress factor and their stress response was characterized 72 h later by a series of physiological, biochemical and molecular approaches. The application of 4-PU30 and MEIA had an obvious effect on the reaction of wheat and einkorn to the applied stresses, leading to the alleviation of oxidative stress damage. Transcriptional activity of genes encoding key ROS-scavenging enzymes such as catalase (CAT), peroxidase (POX), superoxide dismutase (SOD) and glutathione S-transferase (GST) was also assessed to investigate the genetic regulation of the antioxidant systems in both wheat and einkorn seedlings. Differential transcriptional modulation and specific expression patterns of different antioxidant genes were observed in both wheat species, with members of the *POX* and *GST* gene families identified as the most responsive genes. Overall, the

summarized results highlight the positive effect that the applied plant regulators can have on the tolerance of cereals to oxidative stress, providing opportunities to alleviate negative stress effects in future agricultural practices in view of the increasing nutritional needs of the human population.

This research was supported by Bulgarian National Science Fund (BNSF), Grant No KP-06-N56/15.

O-3

Evolutionary insights into β-cyclocitral signaling in *Physcomitrium patens*

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Keywords: apocarotenoids, β-cyclocitral, light stress, stress signalling

Carotenoids are central to plant signaling, serving as precursors to phytohormones like abscisic acid and strigolactones. Among their oxidative derivatives, β-cyclocitral (β-CC) acts as a potent photoxidative stress signal. Produced through β-carotene oxidation in chloroplasts under excessive light (EL), β-CC acts as a retrograde signal and triggers a nuclear detoxification response, protecting cells from oxidative damage. SCL14, a GRAS-domain protein, leads this pathway, activating a cascade of ANAC transcription factors, which in turn induce detoxification enzymes.

Yet, β-CC signaling remains cryptic: a volatile, water-soluble molecule with no known receptor and no biosynthetic mutants for functional studies. To tackle its molecular signaling, we explored its evolutionary origins, investigating *Physcomitrium patens*, a bryophyte that provides a window into early land plant stress responses, offering insights on mechanisms predating vascular plants, which acquired more sofisticated processes to handle photooxidative stress.

Using PAM fluorimetry, we tested β-CC effect on *P. patens* under EL stress. Instead of enhancing protection, β-CC reduced photosynthetic efficiency and increased ROS accumulation. Similarly, the SCL14-regulated detoxification system is incomplete in Physcomitrium and may represent a key transition in β-cyclocitral response across more recent plant lineages. However, its strong transcriptional response, largely overlapping EL repsonse, suggests a conserved but functionally divergent β-CC response in bryophytes, shedding light on the evolutionary trajectory of carotenoid-derived stress signaling. Moreover, we identified a direct and specific effect of β-cyclocitral on photosynthesis, a response absent in Angiosperms.

This divergence suggests an early signaling role that may have been reprogrammed in vascular plants as a proper retrograde signaling to optimize oxidative stress resilience. Further investigation will clarify how this pathway evolved and its broader implications for plant adaptation to terrestrial environments.

This work was financed by the European Union – Next Generation EU, Mission 4, Component 1, CUP D53D23022160001

O-4

The role of FLA7 (*Bradi3g39740*) in salt stress response and plant development in *Brachypodium distachyon*

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Keywords: Brachypodium distachyon, fasciclin-like arabinogalactan protein, plant development

Fasciclin-like arabinogalactans (FLAs) are cell wall glycoproteins belonging to the diverse family of hydroxyproline-rich glycoproteins. They are expressed at various stages of plant development and upregulated in response to stress. In *Arabidopsis thaliana*, the disruption of *FLA4* (also known as *SOS5*) increases salt stress sensitivity, resulting in swollen root cells, while defects in shoot regeneration are exhibited in *fla1* mutants (Acet and Kadıoğlu, 2020; Johnson et al., 2011). However, the functional roles of FLAs, specifically in monocots, remain largely unknown. In *Brachypodium distachyon* Bd21, a model species for temperate C3 grass crops, 23 FLA-encoding genes have been identified. This research explores the role of *FLA7* (*Bradi3g39740*) in plant development and stress response.

The inactivation of the *FLA7* gene resulted in reduced germination efficiency and shorter root length compared to the wild type under salt stress conditions. Proteomic analysis showed similar responses of *fla7* and wild-type

roots in response to the salt stress; however, the upregulation of stress-related genes, such as late embryogenesis abundant proteins (dehydrins) and cupins, was more pronounced in the wild type. Metabolomic analysis revealed perturbations in polyphenols, phytochromes, and amino acids in response to salt stress. Additionally, differences in seed morphology were observed, with *fla7* seeds being shorter and slightly wider than those of the wild type. Seed metabolomics indicated a reduction in polyphenols, jasmonic acid biosynthesis pathway, and glutathione pools, accompanied by an increase in fatty acids content in *fla7*. Immunostaining of the root differentiation zone using various epitopes highlighted changes in cell wall composition, specifically within the endodermis and rhizodermis, in response to salt stress.

Our results demonstrate the crucial role of FLA7 in seed and root development as well as the salt stress response in *B. distachyon*. Ongoing efforts aim to elucidate the molecular mechanisms underlying FLA7 activity.

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O-05

Root carbon shortage leads to mortality of drought-stressed trees

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Keywords: *carbon starvation, forest dieback, hydraulic failure*

Hydraulic failure and carbon starvation have been proposed as possible physiological mechanisms to explain water deficit-induced forest dieback. Hydraulic failure occurs during drought-induced water stress when xylem tension becomes high enough to cause air-seeded embolism, which occludes water transport, exceeding the survival threshold. Soil water deficit leads to stomatal closure, reducing photosynthesis and carbon supply, leading to plant carbon demand exceeding carbon supply. A prolonged imbalance between carbon demand and supply depletes plant carbon storage, resulting in carbon starvation. The carbon starvation (or limitation) hypothesis posits that drought-stressed trees die because their photosynthetic carbon production becomes insufficient to meet minimum survival requirements. Recent studies have shown that a significant carbon shortage exists in the roots of drought-stressed trees. Moreover, hydraulic failure and carbon starvation are likely highly interactive during tree mortality and are not mutually exclusive mechanisms. Building on the interaction between these mechanisms, this talk will explore how root carbon shortages exacerbate tree mortality under drought stress.

O-6

Recovery of photosynthetic activity in resurrection plants *Ramonda serbica* and *Ramonda nathaliae* after freezing-induced desiccation

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Keywords: resurrection plants, ramonda, photosynthesis, recovery, freezing stress

Resurrection plants are increasingly attracting interest due to their unique properties in response to climate change. This group of plants includes also *R. nathaliae* and *R. serbica*, both of which have remarkable resistance to extreme higher and lower temperatures. This study is the first to examine whether these plant species can restore photosynthetic activity after freezing-induced desiccation. The primary goal of this research is to gain a more detailed understanding of the processes that enable these plants to recovery to full functionality after freezing stress in natural conditions. The research was conducted on plant samples of both species collected under natural conditions, where temperatures were lower than -10°C. These plants were then gradually revived under ex situ conditions. Photosynthetic parameters were monitored at intervals of 1, 3, 6, 9, 12, 24, 48, and 72 hours, as well as on the 7th day after the initiation of revival. All photosynthesis and chlorophyll fluorescence parameters were measured. The results indicate that photosynthesis (including assimilation and stomatal conductance) begins to recover within 6 hours of resuscitation. Meanwhile, the quantum efficiency of photosystem II exhibited significant changes during the first hours of revival and was restored only after 24 hours of rehydration. Notable differences were also observed between the two species.

The study suggests that *R. serbica* restores photosynthetic activity much faster than *R. nathaliae*. These findings could be valuable for future molecular research on these species and may also contribute to the application of this knowledge in crop improvement.

This research was supported by the Ministry of Education, Science, Technology and Innovation, Government of Kosovo, project number 2/2282-1.10.

O-7

Elucidate the reality and functionality of NAtural Deep Eutectic Solvents (NADES) formation in desiccation tolerant seeds

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Keywords: desiccation-tolerance, NaDES, seeds

Desiccation tolerance represents one of nature's most extraordinary survival strategies, allowing anhydrobiotes to survive with less than 10% water in their tissues. In orthodox seeds, desiccation tolerance is acquired during dehydration and prior to vitrification, which is initiated at 23% water content. Although the mechanisms governing desiccation tolerance are not fully understood, the involvement of compatible solutes capable of exchanging hydrogen bonds, such as disaccharides, amino acids and organic acids, appears to be a key factor. However, the mode of action of these solutes is not well established. Furthermore, these compounds are increasingly used in green chemistry especially as deep eutectic solvents (DES) ingredients. DES are viscous liquids, formed by mixing hydrogen bond donor and acceptor compounds, widely used especially for natural substance extraction and biocatalysis. Given the natural occurrence of their ingredients and their cell compatibility with biomolecules, the formation of natural DES (NaDES) in living tissue, as a third liquid phase, is an increasingly plausible hypothesis. In seeds, NaDES could solubilize and protect macromolecules during dehydration. However, the presence and the functionality of NaDES in living organisms remains hypothetical and require tangible experimental evidence. Here, we propose concrete elements to support this hypothesis. Thanks to metabolic profiling by GC-MS and LC-UV-MS, we identified putative NaDES ingredients such as sugars, amino acids and organic acids in seed extracts. The molecular interactions and affinities between these ingredients have been detected from the seed extract but also a solution of standards. The colocalization of these reactants and their preferential interactions in seed compartments was pointed out by mass spectrometry imaging (MSI). Based on these results, some physico-chemical properties of reconstituted mixture, such as vitrification, have been established and translated to the physiological environment of seed tissues. This work highlights selected eutectic mixtures, prepared from ingredients that are present, colocalized and interact in seed compartments, whose physico-chemical properties and functionality are compatible with the seed's cellular environment. Their ability to protect macromolecules, such as enzymes, against desiccation and thermal stress will be tested *in vitro*.

O-8

Mitochondrial electron transport mutants modulate drought tolerance in *Arabidopsis*

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Keywords: *Arabidopsis thaliana*, mitochondrial ETC, ROS production, drought tolerance

Adaptation to unfavorable and rapidly changing environmental conditions is one of the most important challenges in crop production today. Although several biological processes are involved in plant adaptation, the role of mitochondria is indispensable. In response to abiotic stress conditions mitochondria regulate important segments of cellular metabolism including respiration and oxidative phosphorylation, redox balance, and reactive oxygen species (ROS) production. In mitochondria, over-reduction of the electron transport chain (mETC) is the primary reason for ROS accumulation, which can be regulated by protecting and stabilizing electron flow. The major sites of ROS production are

Complex I and Complex III of the mETC; therefore the mutations in genes encoding these proteins can play crucial role in stress responses.

To reveal the importance of genes encoding the mETC proteins in stress responses, we analyzed insertion mutants of 13 *Arabidopsis thaliana* genes that encode subunits of Complex I and III. The phenotypes of the mutants were characterized under osmotic, salt and oxidative stress conditions. Morphological alterations and differences in tolerance to drought and salinity were revealed through germination and growth tests, and by complex phenotyping. Two mutants were characterized in detail, with mutations affecting either the Complex I subunit NDUFS8-1, or PPR40, a protein associated with Complex III.

The *ndusf8.2-1* mutant showed tolerance to osmotic and oxidative stress, with lower hydrogen peroxide level and reduced lipid peroxidation rates. Moreover, *ndufs8.2-1* tolerated water deprivation, retained photosynthetic activity better and recovered from severe water stress with higher efficiency than wild type plants. Several mitochondrial functions were altered in the mutant including oxygen consumption, ROS production, ATP and ADP content, as well as the activities of genes encoding alternative oxidase 1A and various alternative NAD(P)H dehydrogenases. Our data revealed that *NDUSF8.2* plays important role in plant stress responses, and that a strong correlation exist between mitochondrial function, photosynthetic activity and ROS homeostasis.

Our previously described *ppr40-1* mutant is hypersensitive to ABA, and its redox balance is altered in the absence of the PPR40 protein. We examined the drought tolerance of *ppr40-1* and found that the mutant was more tolerant to water deprivation than wild-type plants in various growth and development parameters. Additionally, the mutants showed higher relative water content, increased survival rates, reduced level of oxidative damage and more stable photosynthetic performance in water-limited conditions. Fast stomatal closure was observed in *ppr40-1*, demonstrating enhanced water conservation ability that supports the mutant drought-tolerance phenotype. The altered expression of ROS-related genes and changes in H_2O_2 content in the *ppr40-1* mutant could correlated with ABA responses.

These results suggest that certain mitochondrial proteins such as NDUFS8.2 and PPR40 are involved in maintenance of redox homeostasis which can modulate plant responses to water deprivation. Our findings can provide an opportunity to use mitochondrial electron transport genes as potential candidates for improving drought tolerance in crop plants.

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Research data were published in the following papers:

Kant K, Rigó G, Faragó D, Benyó D, Tengölics R, Szabados L, Zsigmond L (2024) Mutation in Arabidopsis mitochondrial Pentatricopeptide repeat 40 gene modulates tolerance to water deficit. Planta 259(4):78.

Zsigmond L, Juhász-Erdélyi A, Valkai I, Aleksza D, Rigó G, Kant K, Szepesi Á, Fiorani F, Körber M, Kovács L, Szabados L (2024) Mitochondrial complex I subunit NDUFS8.2 modulates responses to stresses associated with reduced water availability. Plant Physiol Biochem 208:108-466.

O-9

Responses of intrinsic water-use efficiency and tree growth of sessile oak to climate change in North Hungarian central range

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Keywords: climate change, δ18O, intrinsic water use efficiency, sessile oak, basal area increment

Due to climate change, vitality loss and mortality of forest tree species are increasing. Despite intensive research, the mechanisms involved in drought resilience and survival are still not fully understood.

In this study we investigated the tree growth (basal area increment, BAI), $\delta^{13}C$ derived intrinsic water use efficiency (WUE_i) and $\delta^{18}O$ ratio in tree rings to estimate the long-term variation (1940-2020) in resilience of sessile oak (*Quercus petraea* (Matt.) Liebl.) across four sites in N-Hungary in correlation of climate. In each sites we analyzed trees from different vitality classes as assessed by crown defoliation extent.

WUE_i derived from $\delta^{13}C$ basically exhibited an increasing trend during the study period in high vitality trees. However in trees with declining tree vitality the increase of WUE_i was more moderate in each site. Increasing air CO_2 concentration since 1960 resulted in higher intrinsic water use efficiency (WUE_i) but most synchronously in

trees of high vitality class in all sites. WUE_i positively correlated with BAI, but the strength of relationships between WUE_i and BAI varied across vitality classes and sites. WUE_i positively correlated with $\delta^{18}O$ especially strongly in trees with higher vitality but with declining strength in trees with low vigour.

This study was founded by the NKFIH SNN 125652.

O-10

The root code cracked: Deciphering wheat exudation during drought

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Keywords: root exudates, drought, peptides

To survive and thrive under challenging conditions, plants have developed complex adaptive mechanisms, one of which is the production of a mixture of organic compounds called root exudates, which are actively released by the plant into the surrounding soil. Root exudates play a crucial role in plant-soil-microbe interactions, especially under environmental stress. Drought causes significant changes in the amount and composition of root exudates and has many adverse effects on crops and their associated microbes. However, the precise modulation of the exudation remains poorly understood and genetic factors (species, cultivars) may also contribute to the complexity of the relationship between root exudation and drought response.

Here, we developed a semi-hydroponic system to grow crops under sterile conditions and collect root exudates after water deficit. Metabolomic, peptidomic, proteomic analyses were performed to investigate the impact of drought on the composition of root exudates released by two contrasting drought-responsive wheat cultivars.

Our results showed that drought stress significantly altered the exudate profiles, leading to the release of specific compounds, including carbohydrates, phenolics, amino acids, and proteins.

Some small secreted peptides (SSPs), important signals in plant stress tolerance identified in the wheat genome, were detected for the first time here in drought-related root exudates. Moreover, comparative profiling revealed significant differences in exudate composition between cultivars, suggesting a link to their differential stress responses.

Overall, this research provides valuable insights into plant-soil interactions under drought and contributes to the development of strategies to enhance crop resilience for sustainable agriculture.

This work was supported by the grants PRIN2022 - ROOTEM

O-11

Mutation in a barley strigolactone repressor *HvD53A* impairs photosynthesis but increases drought tolerance

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Keywords: barley, repressor, strigolactone

Strigolactones (SL) are the youngest group of plant hormones, initially discovered for their role as signaling molecules in interactions with root-parasitic plants. Further research confirmed their involvement in shoot branching, root architecture, as well as regulation of plant stress responses. This study examined the effect of HvD53A, a barley SL repressor, on plant growth and drought tolerance. The *hvd53a.f* mutant, identified by TILLING, showed reduced shoot branching, increased height, and delayed flowering. Chloroplast ultrastructure analysis revealed smaller chloroplasts and fewer grana stacks, which reduced the photosynthetic efficiency. RNA-seq analysis linked differentially expressed genes (DEGs) in *hvd53a.f* to antioxydation and stress responses. Comparative transcriptomic analysis of *hvd14.d*, an SL-insensitive mutant, revealed that both SL mutants share at the same time common and distinct regulatory pathways, with specific transcription factors (TFs) mediating SL-dependent responses. Our results revealed a strong connection between the SL-signalling pathway and circadian clock components, which might explain the

contrasting differences in the phenotypes of *hvd53a.f* and *hvd14.d* mutants. Among these, CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) emerges as a potential SL-responsive TF, playing a key role in regulating tillering.

Under drought stress, *hvd53a.f* exhibited enhanced tolerance, maintaining higher relative water content, lower chlorophyll degradation, and stable, but reduced photosynthetic efficiency. We conclude that *hvd53a.f* plants exhibit greater drought tolerance at the cost of lowering photosynthetic efficiency, while maintaining a stable low level. Transcriptome analysis revealed a shift in drought response with *hvd53a.f* activating protective mechanisms, such as dehydrin and aquaporin accumulation. Moreover we identified potential SL-related TF, JUNGBRUNNEN 1 (JUB1) regulating the expression of genes involved in water deficit regulation and antioxidation processes. In contrast, *hvd14.d* displayed increased drought sensitivity. Overall, our findings link SL signalling, photosynthesis, and drought adaptation, identifying HvD53A as a key player in stress resilience, and offering potential targets for crop improvement.

This work was supported by the grants National Science Centre, Poland (2018/31/F/NZ2/03848).

■

O-12

Thermotolerance of natural *Arabidopsis* accessions from Catalonia growing under salinity

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Keywords: thermotolerance, salinity, natural variation

Climate change is increasing the incidence of abiotic and biotic stresses, with salinity and short-term heat stress posing major global challenges. These stresses affect plant growth, productivity, metabolism, reproduction, and photosynthesis. While individual stressors are significant, plants in nature often face multiple and more complex stress combinations that can trigger unpredictable responses. Genetic diversity plays a key role in helping plants adapt to diverse environments and changing climates. The present study aims to investigate the natural variation in the thermotolerance of several Arabidopsis thaliana demes from the Catalonia region grown under salinity. A first screening of 20 demes identified those with contrasting thermotolerance phenotypes. Once selected, the responses under combined heat and salt stress were examined with a particular focus on identifying key genetic factors and tolerance mechanisms during primary photosynthetic processes. Results showed that demes such A1 and A5 presented an enhanced activation of tolerance mechanisms and a lower decline in photosynthetic metabolism under heat stress, compared with the reference accession Col-0. Gene expression analysis conducted before and after the heat shock treatment (42° for 1 hour) showed that in A1 and A5 demes the relative expression of the cytosolic heat shock protein Hsps101 was higher than in other demes under both control (0 mM NaCl) or saline conditions (50 mM NaCl). However, the gene expression of Hsps101 and salt regulators such SOS1 and NHX1 was significantly reduced when plants grew under salinity, showing in fact the complexity of the response in case of multiple stressors. This research elucidates potential mechanisms that plants employ to cope with heat and salt stress, mechanisms that need further attention to improve crop resilience in the face of challenging environmental conditions

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O-13

Devising strategies to incorporate halophytes into the farming systems

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Keywords: halophytes, intercropping, micropropagation, sequential cropping

Most arable lands in Mediterranean countries are located in arid and semiarid regions, where water and soil salinity, water shortage and nutrients deficiency in soils are the major constraints affecting food and fodder production. In this context, halophytes emerge as alternative cash crops to be used in sustainable saline production systems, due to their ability to cope with soil and water salinization and to restore biodiversity. The overall objective of our research is to develop sustainable and environmentally friendly new farming and producing systems based on the use of halophytes. For this purpose, different strategies are being pursued:

(a) Determine the feasibility of mixed cultivation between halophyte and a cash crop: Intercropping and sequential cropping between tomato (*Solanum lycopersicum* L.) and the halophyte *Arthrocaulon macrostachyum* were conducted in a greenhouse under moderately saline conditions. The results showed that both agronomic managements enhanced crop performance while reduced soil salinity. Sequential cropping improved nutrient homeostasis in tomato plants, which was reflected on an increased fruit production, while intercropping enhanced photosynthesis. Moreover, both crop managements triggered a mild oxidative stress in tomato plants, which could be related with the establishment of adaptive responses. These data support the sustainability of practices based on the cultivation of halophytes.

(b) Implement micropropagation of halophytes for the selection of elite clones: Here, we achieved efficient micropropagation of *A. macrostachyum* and two species from *Salicornia* genus (*Salicornia lagascae* and *Salicornia europaea*). Superior clones were selected based on salinity tolerance, which were then rooted and acclimatized to *ex vitro* conditions. A comprehensive characterization including determination of oxidative stress parameters, photosynthesis efficiency and mineral nutrient contents was done during this process. This approach provides a solid *in vitro* platform for the production of elite halophyte genotypes for ulterior uses, independently on seasonal variations and with prospect of scaling up.

(c) Determine whether *in vitro*-propagated halophytes outperform wild halophytes in salt-remediation capacity under *ex vitro* conditions: We compared the physiological and biochemical responses of selected clones of *A. macrostachyum* obtained *in vitro* and their wild counterparts to high salinity level, growing under greenhouse conditions. *In vitro*-derived clones displayed a superior biomass production, salt accumulation capability and photosynthetic performance than the wild plants. On the other hand, a higher incidence of salinity-related oxidative stress in the wild halophytes in comparison to the clones was observed. This represents the first *ex vitro* evaluation of halophyte clones selected by means of micropropagation, and validates the potential of this clonal material for desalinization purposes.

O-14

Long-term high temperatures affect seed maturation and seed coat integrity in *Brassica napus*

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Keywords: *Brassica napus*, long-term high temperature, cell wall, pectin, seed coat rupture, seed development

Global rise in temperature puts the development and yield of many important staple crops at substantial risk, threatening food security. An agronomically important crop, *Brassica napus,* is the world's second most widely produced vegetable oil crop. We characterised and studied the vegetative and reproductive development of the three spring cultivars DH12075, Topas, and Westar grown at prolonged high temperatures(32°C). At 32°C, *B. napus* undergoes thermomorphogenesis based on the analysis of the plant's vegetative growth, seed yield, and embryo development. Prolonged growth at elevated temperatures negatively impacts the fertilisation rate, embryo development, seed viability, and yield and accelerates embryo development. During seed maturation, plants developed under 32°C undergo seed

coat rupture (SCR) with or without embryo protrusion. The occurrence of SCR in the cultivar Topas was around fifty per cent, severely impacting the yield quality. Further examination of Topas seeds using hormonal, transcriptomic and seed size studies strengthens the assumption that dormancy-related factors do not regulate SCR. Instead, it is controlled by the biophysical aspects of the communication between the seed coat and accelerated embryo development at the seed maturation stage, which is influenced by high temperature. This hypothesis was backed by the Enrichment of demethylesterified pectin subunits in the seed coat cell walls of seeds developed at high temperatures. Such a modification could affect the strength of the developing seed coat. The SCR phenotype in Topas grown at high temperatures is caused by the accelerated embryo development, which, when coupled with the heat stress-dependent reduction in the thickness of seed coat layers and untimely cell wall modification, is found to be the cause of the SCR phenotype in Topas grown at high temperatures. Our research provides valuable information for future plant breeders to account for the effects of mechanical properties during seed development, which is misguided by the growth of *B. napus* at elevated temperatures.

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O-15

Impact of GenX on the physiology of *Solanum lycopersicum*: Implications for agriculture and environmental safety

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Keywords: PFAS contamination, GenX, agricultural impact, photosynthesis, stress response, *Solanum lycopersicum*

The widespread use of per- and polyfluoroalkyl substances (PFAS) in industrial applications has resulted in significant environmental contamination due to their high persistence and mobility. In response to these concerns, alternative compounds such as hexafluoropropylene oxide dimer acid (HFPO-DA or GenX) have been introduced. However, the high-water solubility of GenX increases its potential for dispersion and pollution of agricultural ecosystems. Building upon previous research on *Zea mays*, this study investigates the impact of GenX on the morphology and physiology of *Solanum lycopersicum* (*cv. Florida Petite*), a C3 plant, with specific focus on growth, photosynthesis, transpiration, and broader biochemical responses. Five-week-old tomato plants were grown hydroponically and exposed to three GenX concentrations (10, 100, and 1000 µg/L) for 14 days.

Analysis of chlorophyll fluorescence revealed the inhibition of electron transport, which led to the reduction of photosynthetic efficiency and the promotion of energy dissipation mechanisms. Additionally, gas exchange measurements indicated significant reductions in stomatal conductance and CO_2 fixation, suggesting impaired water uptake and dysregulation in carbon metabolism. Indeed, transmission electron microscopy (TEM) and biochemical assays confirmed GenX concentration-dependent accumulations of starch in chloroplasts and upregulated antioxidant activities, linking altered carbohydrates metabolism to stress responses and compromised photosynthetic performances. Besides, molecular and biochemical stress markers corresponded to significant growth deficits, including reduced root and aerial development, highlighting GenX impact on plant health and productivity. Ongoing proteomic analyses are being conducted to identify key regulatory proteins affected by the exposure. GenX accumulation in leaves and fruits, also indicates the need for a reassessment of PFAS substitutes' safety in agricultural settings, especially in relation to human health risks. Future research should focus on long-term effects on plants, on possible soil microbiome interactions, and on mitigation strategies like bio- and phytoremediation, to uphold proper environmental and food security standards, posing the basis for future regulation plans.

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O-16

Chronic ionizing radiation impact on pathogen resistance in aquatic plants

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Keywords: Chornobyl zone, *Phragmites australis*, proteome profiling, phytopathogen assay, protein carbonylation

Chronic ionizing radiation may trigger multiple signaling cascades in plants that induce molecular and cellular changes. It may cause water radiolysis, consequently generating excessive DNA damage and reactive oxygen species. In response, plants may adapt by synthesizing defense proteins and metabolites, including antioxidants. Moreover, chronic radiation may affect plant immunity to subsequent biotic stress (Mishra et al., 2024). Aquatic ecosystems in Chornobyl zone, a major radiological disaster site, are contaminated by harmful radionuclides. Our study focused on explaining the biochemical mechanisms responsible for the susceptibility of a wild aquatic plant (common reed, *Phragmites australis*) grown in Chornobyl zone to biotic stress. The fungal infection assay indicated that life in a radionuclide-contaminated environment compromised plant immunity. Proteome profiling identified 1,867 proteins and we selected several dozen proteins with consistently higher and lower abundance in the samples from the litoral of contaminated lakes by hierarchical clustering. Discordant expression of coding genes pointed to posttranscriptional regulation. Proteins that accumulated in reed upon chronic irradiation suggested a radioresistant phenotype with effective protection against reactive carbonyls. Simultaneously, proteins that depleted in plants collected from litoral of radiologically contaminated lakes indicated worse stress resilience and enhanced susceptibility to biotic agents. Furthermore, quantifying antioxidant enzyme activities and carbonylated proteins rebutted the idea of substantial oxidative stress in chronically irradiated plants. We advocate the necessity to consider increased pathogen sensitivity while developing policies for the management of radionuclide-contaminated areas.

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O-17

High light as a tool for improving thermotolerance of rice plants through elevated cytokinin levels

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Keywords: cytokinins, heat stress, high light intensity, *Oryza sativa*, phytohormones

Cytokinins are important phytohormones regulating plant growth and development. Their levels often decrease during abiotic stress to stop growth. This reduction also occurs when plants are under heat stress. However, if we artificially increase cytokinin levels, plant stress tolerance increases, similarly to acclimation by mildly elevated temperatures. Cytokinin levels can be elevated by high light affecting thermotolerance, too. We compared the effect of high light on rice plants exposed to heat stress targeted to whole plants, leaves or roots and identified positive impact of light intensity on thermotolerance through cytokinins and location of their synthesis.

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O-18

Transcription fidelity control contributes to heat stress survival in *A. thaliana*

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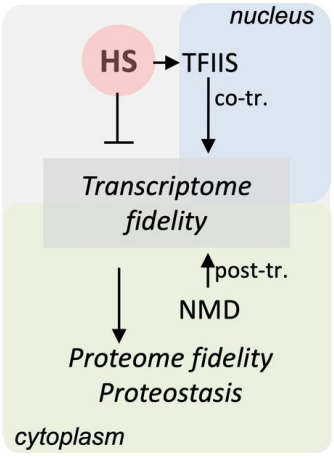
Transcriptional fidelity is essential to maintain the integrity of genetic information. Although it is well characterised in yeast and metazoan, regulation of transcriptional fidelity in plants remains elusive. By employing genetic, molecular biology and deep sequencing tools, we explored transcriptional fidelity and *trans* factors that control it in *Arabidopsis thaliana*. Using circle-sequencing assay we analysed the error landscape of transcriptome at single nucleotide depth under ambient and heat-stress conditions in wild-type and different quality control mutants. We found that frequency of nucleotide misincorporations and insertions is significantly elevated under heat stress, and that nucleotide imbalance also leads to error-prone transcription. We demonstrate that RNA polymerase II associated elongation cofactor TFIIS ensures low transcriptional error rates, which explains why this fidelity factor is essential under both heat-stress and nucleotide mis-incorporation stress conditions. Moreover, we found that nonsense-mediated mRNA decay (NMD) cytoplasmic surveillance system, that degrades premature termination codon-containing aberrant transcripts, also play a role in heat stress response. Besides fidelity safeguarding, NMD and TFIIS cooperatively regulate several other aspects of transcriptome, including alternative splicing to maintain proteostasis. Therefore, the interplay between these RNA surveillance systems safeguards the correct genetic information flow and is critical for developmental regulation and heat-stress adaptation in plants.



O-19

Heat tolerance and functional traits of Mediterranean tree species: A comparation between deciduous and evergreen species

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Keywords: heat stress, heatwaves, urban environment, functional traits, abiotic stress

The Mediterranean basin and its urban areas have long been characterized by vulnerability and delicate balances, due to historical anthropization. In recent years, there has been an increasing frequency and intensity of anomalous heatwaves, which have serious consequences for tree species, both deciduous and evergreen. It is therefore essential to clarify the functional and morphological effects of heatwaves on woody species and investigate the differences between deciduous and evergreen, considering the specific characteristics of each. Additionally, it is important to understand the species heat tolerance thresholds along with the correlation between these and the morphological and physiological traits of plants, with particular emphasis on those associated with heat stress resistance.

Three Mediterranean species were selected for the study: two deciduous species, *Acer campestre* and *Fraxinus ornus*, and one evergreen, *Quercus ilex* in order to evaluate their tolerance to heat stress; the indices *Tcrit* (the critical temperature that causes initial damage, 15%, to the maximum quantum yield of PSII, Fv/Fm) and *T50* (the temperature that causes 50% damage to the maximum quantum yield of PSII, Fv/Fm) were determined for this purpose. The study was conducted during the summer of 2024, which saw extremely high heat peaks, affecting both minimum and maximum temperatures mostly in July. Significant differences were observed both between the thermal tolerance thresholds of deciduous and evergreen species, and in the analysis of their correlation with functional traits. The evergreen species exhibited greater heat tolerance, mainly relying on structural and morphological leaf adjustments to maintain maximum PSII quantum yield even at high temperatures. The deciduous species, on the other hand, showed a more functional adaptation, but with a lower

capacity to tolerate high temperatures. Among the indices considered, *Tcrit* was the most correlated with the morphological and functional traits, making it a sensitive and predictive variable for assessing heat tolerance in species. This study provides valuable insights for evaluating tree tolerance to heatwaves and predicting their functional responses in urban environments. Investigating the electrolyte leakage and, more generally, the behavior of cell membranes in response to heatwaves could provide a valuable direction for further study and help confirm the results obtained thus far.

In summary, the findings of this research are useful for better managing urban green spaces, aiding in the selection of species best suited to cope with rising temperatures in Mediterranean cities.

O-20

Physiological and biochemical changes occurring during deacclimation of oilseed rape – relation to frost tolerance

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Keywords: oilseed rape, cold acclimation, deacclimation

Winter crops acclimate to frost during the process of cold acclimation mainly in autumn months, when plants are exposed to low but non-freezing temperatures that is connected to specific metabolic adjustments. Due to climate changes periods of warm break with higher temperatures are becoming more frequent in late autumn and winter. It disturb the natural processes of cold acclimation of winter crops and leads to the deacclimation that can make plants more susceptible to frost. Deacclimation can even result in a resumption of plant growth, thus becoming a serious problem for agriculture. The aim of these studies was to investigate the physiological, biochemical and molcular changes that accompany deacclimation process in the economically important winter oilseed rape plants. The experimental model included plants that were non-acclimated, cold acclimated (at 4 °C, 3 weeks) and deacclimated (7 days at 16 °C/9 °C). The chemical composition of the leaves, which was measured using FT-Raman spectroscopy clearly confirmed the metabolic differences between the cold-acclimated and deacclimated plants. Frost tolerance was decreased after deacclimation in comparison to cold acclimated plants, which was accompanied by: (1) decrease in the content of water soluble sugars, (2) decrease in the activity of sucrose synthase and sucrose phosphate synthase (SUS and SPS) enzymes and (3) increase the relative expression of sugar transporters *BnSUC1* and *BnSWEET11*. As a result of deacclimation there was observed increased photosynthetic activity particularly increase in PSII efficiency. Deacclimation decreased of the Rubisco activity. As a result of this process, there was an increase in the expression of the *BnRcA* and *BnRbcS* genes and the accumulation of the RbcL protein. We have also demonstrated the changes in the water management occurring as a result of the deacclimation process. It was observed increase of leaf relative water content and the osmotic potential, decrease in the accumulation of the aquaporin protein BnPIP1 and BnPIP2 and increase in the relative expression of *BnPIP1* and *BnPIP2* aquaporin transcript. Moreover, deacclimation also decreased the accumulation of protective proteins from a group of heat shock proteins (HSP) and influenced on the hormonal homeostasis.

In summary, deacclimation partially or completely reversed the changes that occurred in the plants during cold acclimation. Deaclimation-induced changes in regulation of sugar metabolism and water management seem to be among the most important factors that can contribute to decreased frost tolerance of deacclimated oilseed rape.

O-21

Heavy metal accumulation and tolerance in Szarvasi-1 energy grass

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Keywords: heavy metal, ionomic pattern, phytoremediation

Biomass plants such as Szarvasi-1 energy grass (*Thinopyrum obtusiflorum* syn. *Elytrigia elongata*, *Elymus elongatus* subsp. *ponticus* cv Szarvasi-1) have been developed to serve as a source of bioenergy (biofuel, biogas and combustion). However, fast growing plants

developing a large above and below ground biomass may also be suitable for phytoremediation of soils contaminated with heavy metals. Based on previous results obtained in hydroponically grown plants we have conducted experiments to reveal the Cd, Cu and Pb accumulation and tolerance of Szarvasi-1 plants grown in soil cultures. The soil was amended and incubated with 5/10/50 mg kg^{-1} Cd, 100/500/1000 mg kg^{-1} Cu or Pb and a mixture of the three metals in increasing concentration series (1/2/3). Szarvasi-1 plants grown for two months have been counted, physiological and elemental data were collected and evaluated with multivariate analysis.

The number of tillers per pot, total shoot dry weight, chlorophyll content (Chl) and photochemical reflectance index (PRI) decreased in the Pb<Cu~Cd order whereas the relative water content and malondialdehyde content of the shoots were stable across the treatments. In the concentration series of the mixed metal treatments, the growth variables, Chl and PRI decreased sharply whereas malondialdehyde increased. The accumulation of heavy metals decreased in the following order Cd>Cu>Pb in both single and mixed treatments but in the latter the proportion of Pb slightly increased. The nutrient and trace elements in the shoot biomass showed detectable changes characteristic to the treatments applied.

It has been concluded that Szarvasi-1 plants are less sensitive to low concentration of single metal contamination but higher concentrations and mixed treatments reveal its tolerance limits.

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O-22

Pectinous cell wall thickenings formation - a widespread defence strategy in plants growing on substrates polluted with toxic trace metals and metalloids

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Keywords: vesicular transport, cell wall remodelling, phytoremediation

The plant cell wall (CW), besides the vacuole, is one of the main compartments for toxic elements (TMs) sequestration. The results of our research revealed that both the composition and the structure of CW can be remodelled in plants exposed to TMs. It often leads to CW thickenings (CWTs) formation what we detected in diverse plant species and cell types differing in the type of growth, e.g. in the roots of *Arabidopsis thaliana* and *Populus tremula x tremuloides* and *Funaria hygrometrica* protonemata exposed to a single toxic metal, e.g. Pb, under laboratory conditions, as well as in cells of plants growing on mining sludge polluted with many TMs, such as: As, Cu, Pb, Cd, Hg, Zn, e.g. in roots and leaves of *Acer platanoides* and roots of *Tilia cordata.* Importantly, all analysed CWTs commonly showed particularly high concentration of TMs. It was probably related to the high level of the low-methylesterified pectins (recognized by LM19 antibody - LM19-P) – pectin fraction able to bind and immobilize TMs ions. The colocalization of LM19-P and TMs in CW thickenings was visualized in 3D by electron tomography and additionally confirmed by EDS X-ray microanalysis connected with transmission electron microscopy (EDS X-ray–TEM).

Together with CWTs formation, we detected the intensification of vesicular transport in protoplasts. The cargo of the transporting vesicles (Vs) was Pb deposits and LM19-P. Presence of these two components, in the Vs, was evidently demonstrated in root apices cells of *A. thaliana* exposed to Pb, thanks to use of the high

pressure freezing and freeze substitution technique. This technique allowed us to preserve the biological membranes in the samples and use the immunogold technique for identification of LM19-P within the Vs. Presence of these two components in the Vs we also confirmed, by EDS X-ray-TEM. Importantly, numerous Vs transporting Pb and LM19-P, occurred in the vicinity of the CWTs.

To sum up, formation of CWTs is a widespread defence strategy functioning in diverse plant species to cope with one toxic metal as well as many TMs, present in the substrate. Formation of CWTs increases the CW capacity for TMs sequestration and may underly plant tolerance to TMs. Furthermore, accumulation of the numerous Pb- and LM19-P-containing Vs, near CWTs, suggests their involvement in the thickenings formation.

It is worth noting, that intensification of the mechanisms, naturally present in plants, such as CWs remodelling, that increase the capacity for metal accumulation and perhaps tolerance, provides new opportunities for enhancing plant efficiency for phytoremediation.

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O-23

Evaluation of the sensitivity to inorganic arsenic of carnaroli rice plant (*Oryza sativa* L.)

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Keywords: inorganic arsenic, rice, Carnaroli variety

Italian rice contributes to more than one-half of the European production, with the provinces of Vercelli, Pavia, Novara, and Milan having the largest number of paddy fields for its cultivation. Carnaroli (*Oryza sativa* subsp. japonica) is among the most cultivated and marketed Italian rice varieties, exported worldwide thanks to the high nutritional and organoleptic qualities and cooking seal of its grains, as well as good yield. It originated in Lombardy 80 years ago from a breeding between Vialone and Lencino varieties [1]. However, it is still an understudied variety in relation to tolerance to environmental stresses, particularly arsenic (As).

Arsenic contamination has become a serious environmental issue worldwide and rice is one of the main routes through which As reaches humans. Indeed, rice plants are more efficient at absorbing As than other cereal crops, particularly when As is present in its inorganic forms, arsenite (As^{III}) and arsenate (As^{V}), with As^{III} being more toxic than As^{V}. Inorganic As (iAs) has been classified as Group 1 carcinogens by IARC, and the European Commission has recently set new limits (Commission Regulation EU 2023/465) to reduce its presence in rice-based foods, as a measure to fight cancer and other adverse effects in humans. The flooded cultivation practice promotes the accumulation of iAs in rice roots, altering their development and hormonal homeostasis [2], often leading to phytotoxicity. The As presence in paddy fields points up a global urgent need to select rice varieties able to exclude As from the grains.

This research aims to deepen knowledge on As^{III} and As^{V} toxicity on the development of different rice genotypes with special attention to Carnaroli. Rice plantlets were grown *in vitro* to reduce and control the effect of abiotic factors, and analyzed at the morphological, cytohistological, and chemical levels. The responses of Carnaroli to treatments with iAs, added at toxic concentrations to the culture media, were compared with those of the As-sensitive Zhonghua 11 [3].

Our first results show that Carnaroli exhibited low sensitivity to iAs, although its root uptake of As was higher than in Zhonghua 11. Only the treatment with As^{III} caused a significant reduction in lateral root (LR) production compared to the Control in Carnaroli, whereas this decrease was significantly more pronounced, and with both the iAs forms, in Zhonghua 11. These results were confirmed by histological analysis of the adventitious roots, which are the main component of rice root system, where anomalies in development and protrusion of their LRs were detected. Further analyses are underway to better understand the different effects of As^{III} and As^{V} on rice plant development, and the possible mechanisms which allow different rice genotypes to tolerate or not As toxicity.

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O-24

Modulation of cadmium stress responses in wheat by putrescine pre-treatment under blue and white light

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Blue light is an essential environmental factor that regulates various plant functions, including morphology, photosynthesis, and both primary and secondary metabolism. While some studies suggest that blue light may enhance stress tolerance, its precise mechanisms remain unclear. Polyamines are well known as being involved in stress adaptation, but their interactions with light dependent processes are not well comprehended. Polyamine metabolism could be influenced by light quality, which might affect its relationship with other protective compounds. According to these, the main question of the present work was that whether blue light induces different responses during Cd stress, especially which are related to polyamine metabolism, and whether it may be able to modify the protective effect of exogenous putrescine compared to white light conditions in wheat. The results indicated that Cd stress was less severe under blue light than under white light. Metabolite and gene expression levels were independently affected by blue light, which resulted in reduced Cd uptake, phytochelatin content, and higher levels of conjugated polyamines. Pre-treatment with putrescine provided protection, particularly under white light, and emphasized differences between the effects of blue and white light under Cd stress–especially in phytochelatin synthesis, polyamine metabolism, and the accumulation of phenolic compounds and plant hormones. B light exerted stronger effects on methylation than PUT, while PUT treatment induced more alterations under W light. The gene ontology study revealed that differentially methylated genes are involved in a wide range of cellular processes, such as stress responses, hormone signalling and stress responses. Our results indicate that blue light increases Cd tolerance in wheat and changes the defense mechanisms, particularly when putrescine is present in excess.

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■

O-25

Exploiting somaclonal variability to increase drought stress tolerance in grapevine

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Keywords: *Vitis* Spp., water stress, somatic embryogenesis, genetic variability, gas exchange

Global warming has enhanced the frequency and severity of drought events, hence calling for a better management of water resources in the vineyard and for an improvement of breeding platforms. Somatic embryogenesis (SE) is a morphogenetic process in which the plant regenerative potential is exploited to replicate a whole organism starting from somatic explants. Moreover, SE process and in *vitro* culture can generate somaclonal variability, i.e. genetic variability resulting from gene mutations, changes in epigenetic marks, or phenotypic alterations, which can represent a powerful green biotechnological tool for genetic improvement purposes. The aim of this work was to deepen the somaclonal variation phenomenon and demonstrate whether vines *in vitro* regenerated through SE, namely somaclones, can tolerate water deprivation better than the mother plant. Physiological trials of water stress and recovery were conducted on eleven somaclone lines of *Vitis vinifera* "Nebbiolo" CVT 185. During the experiments, dynamic changes in the main eco-physiological parameters (gas exchange and stem water potential) were evaluated daily on target somaclones compared to the mother plant. Alterations in biometric and anatomical traits and in xylem hydraulic conductivity were also inspected. The observed responses were further deepened by analyzing differences in the accumulation of defense secondary metabolites, non-structural carbohydrates (NSC), starch, osmoprotectant solutes, hormones and in the transcription of stress-responsive genes. In parallel, sequencing analyses of the

genomes of the best and worst performing lines are ongoing to inspect genetic alterations potentially affecting specific physiological modifications. The integration of physiological, biochemical, and molecular data proved that "Nebbiolo" somaclones are more tolerant to drought. The integration of physiological, biochemical and molecular data proved that grapevine somaclones are more tolerant to drought. The exploitation of somaclonal variability can therefore represent an effective and ready-to-use genetic improvement approach for implementing clonal selection and breeding programs in grapevine.

This work was supported by the Agritech National Research Center and received funding from the European Union through the Next-Generation EU programme (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, grant number CN00000022), and by the Shield4Grape: Breeding and integrated pest management strategies to reduce reliance on chemical pesticides in grapevine" project, funded by the European Union through the Horizon Europe Research and Innovation Programme (Grant number 101135088). Views and opinions expressed are, however, those of the authors only. Neither the European Union nor the granting authority nor the European Commission can be held responsible for them.

O-26

Genetic and physiological control of early vigor and transpiration efficiency in rice

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Keywords: rice, early vigor, transpiration efficiency, Farnesyl Diphosphate Synthase

In the current context of water scarcity and global warming, the transition of rice cultivation from anaerobic to aerobic system is crucial to reduce water consumption and greenhouse gas emissions. Early vigor to better compete with weeds and transpiration efficiency (TE) for improved water use are two target traits for breeding new varieties better adapted to aerobic rice agrosystems. We previously identified a genetic locus located on chromosome 5 involved in the control of these traits in African rice (*Oryza glaberrima*) using association genetics. This locus colocalized with QTL previously reported for early vigor and lateral root development in *O. sativa* (Cui *et al*., 2002; Dinh *et al*., 2023). A gene named *OgFPS1* encoding the Farnesyl Diphosphate Synthase 1 was identified as an interesting candidate. Here, we further characterized the role of this locus in the control of early vigor and TE and investigated the function of *FPS1*. To this end, we first performed a Linkage Disequilibrium analysis to define genetic locus into a QTL region and identify different haplotypes for this QTL. A 109 kb QTL region containing 23 candidate genes and two major haplotypes were identified. Expression studies in 8 rice genotypes belonging to the two main haplotypes showed that *OgFPS1* was the only gene significantly more expressed in leaves and roots of the haplotype associated with greater vigor and TE. In parallel, we found that the *Arabidopsis thaliana fps1* mutant shows defects in early vigor and TE. Altogether, these results suggest that *OgFPS1* and his homologous *AtFPS1*, are potentially involved in the control of these traits. Ongoing work aims to validate the QTL region using a biparental population, while examining the functional role of *FPS1* in early vigor and TE through gene editing in *O. sativa*.

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O-27

Using molecular markers to combine freezing tolerance and grain quality in a durum wheat breeding program

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Keywords: durum wheat, freezing tolerance, GWAS, QTNs, KASP markers

Durum wheat (*Triticum durum* Desf.) production is increasingly threatened by climate change, while global pasta consumption continues to grow. To keep up with rising demand and ensure a sustainable pasta supply chain, it is crucial to develop resilient varieties with improved grain quality and stress tolerance. In this study, 250 durum wheat accessions, comprising both winter and spring types, were analyzed. The selection included genetic materials from Eastern Europe (i.e., Russia and Hungary) adapted to cold climates, as well as varieties grown in Southern Europe (i.e., Italy and France) that perform well in hot, dry conditions and offer good grain quality. The main goal was to identify molecular markers linked to important agronomic traits, supporting new breeding programs to develop durum wheat varieties that are both resilient to low temperatures and high quality. Genotyping was performed using a 25K Infinium iSelect SNP array, while phenotypic evaluations were carried out in experimental field trials over two consecutive growing seasons in Italy and Russia to assess the freezing tolerance and quality performance of the genetic materials. Our genome-wide association analysis (GWAS) revealed six significant QTNs linked to freezing tolerance on chromosomes 1B, 2B, 4A, 5A, 5B, and 7A, with the QTNs on chromosomes 5A and 5B together accounting for 27.2% of the total phenotypic variance. In addition, three QTNs associated with wet gluten content were mapped to chromosomes 1B and 5B. To support marker-assisted selection, two QTNs were successfully converted into Kompetitive Allele Specific PCR (KASP) markers. These markers were then used to select durum wheat lines that combined freezing tolerance and high gluten quality within three BC_1F_2 populations obtained by crossing genotypes selected through a parental selection approach. The validation conducted within a real breeding program provides a solid basis for developing climate-resilient durum wheat varieties, ideally suited to future environmental challenges and/or expanding the cultivation area.

O-28

Landscape and regulation of lncRNAs in premature and developmental leaf senescence: A transcriptomic analysis in barley

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Keywords: barley, leaf, long non-coding RNA, developmental senesence, premature senesence

Long non-coding RNAs (lncRNAs) have emerged as pivotal regulators of diverse developmental processes and stress tolerance in plants. Recent investigations have revealed the involvement of lncRNAs in regulating leaf senescence; however, the molecular mechanisms underlying lncRNA-mediated onset and progression of leaf senescence remain poorly understood. In this study, we utilised RNA-Seq to investigate the landscape and regulation of lncRNAs in barley leaves during dark-induced leaf senescence (DILS) and developmental leaf senescence (DLS), aiming to improve our understanding of these processes in this major cereal crop.

We identified 72, 1290, and 2770 differentially expressed lncRNAs (DE-lncRNAs) during day-4, day-7, and day-10, respectively, in DILS, and four significant DE-lncRNAs in DLS. The target genes for these DE-lncRNAs were predicted (cis- and trans-manner), and the potential regulatory network of lncRNAs and their target genes was constructed. The target genes were involved in critical processes such as response to stress, cell redox homeostasis, transmembrane transport, protein folding and nitrogen compound transport. In this study, we report the first identification and characterisation of leaf senescence-associated lncRNAs in the barley genome, offering important insights for both barley and broader crop research. Our results are valuable for gaining deeper insights into the molecular mechanisms underlying leaf senescence, a complex and tightly regulated developmental process with important implications for crop improvement.

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O-29

Building the edit suite: Foundational steps in hemp CRISPR technology

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Keywords: hemp, transformation, CRISPR

Hemp *(Cannabis sativa)* is a vital global crop, renowned for its adaptability, versatility, and rich history. However, unlocking the plant's full genetic potential for targeted breeding and sustainable agriculture requires overcoming the inherent challenges of its heterozygous nature and limited application of genetic techniques. While CRISPR has transformed gene editing in traditional crops, its application in hemp remains underdeveloped. This research aims to bridge this gap by establishing CRISPR-based genome editing techniques suitable for hemp, with the goal of advancing both basic research and applied breeding programs. Our approach involves developing a streamlined transformation process for stable gene editing in hemp. We use transformation methods *Agrobacterium*- based targeting hypocotyl explants in combination with RUBY, a robust reporter system for visual confirmation of successful transformations. Following transformation, rapid regeneration of both shoots and roots was observed at the hypocotyl cut sites within three-four days. Expression of the RUBY reporter was visually confirmed in the regenerated plant tissue. Initial observations suggest a mosaic expression pattern, with signal intensity increasing as the plant develops. These regenerated plants are currently undergoing incubation and will be subsequently transferred to soil for maturation and seed production. This work represents a crucial step towards establishing reliable genetic transformation protocols in hemp, enabling efficient CRISPR/Cas-mediated genome editing and accelerating targeted trait improvement research.

O-30

Validation of the DRO1 gene in tomato under drought stress: Insights into root architecture and drought adaptation

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Keywords: root architecture, drought, CRISPR-Cas9

Drought stress severely limits crop productivity, highlighting the need for strategies to improve water use efficiency while maintaining yield stability. Optimizing root architecture, particularly through genes like *Deeper Rooting 1 (DRO1)*, offers a climate-resilient approach to enhance drought adaptation. *DRO1* has been identified as a key quantitative trait locus (QTL) in rice and Arabidopsis, promoting deeper root growth and access to groundwater during drought. However, root traits like angle and length are species-specific, underscoring the need to explore this gene's role in other crops. This work aims to functionally characterize *DRO1*-mediated root architecture in tomato (*Solanum lycopersicum L.*), a globally important crop. Using an omics-driven approach combining phenomics and genomics, the project investigates the spatio-temporal regulation of *DRO1* and its role in shaping root architecture under drought in the Micro-Tom cultivar.

Root system analysis, including scanning and WinRHIZO, revealed a decrease in root length and very fine root fraction after 30 days of drought, while the root mass ratio increased, indicating a shift in resource allocation.

Imaging techniques showed stable root surface area and fineness across time points. Near-infrared (NIR) imaging on a LemnaTec 3D Scanalyzer confirmed reduced water content in epigeal part of drought-stressed plants, as indicated by increased reflectance. These findings highlight key root functional traits and spectral indices for assessing drought adaptation.

qPCR analysis showed that *SlDRO1* expression in roots remained stable at early stages but increased significantly after 20 days of drought. In contrast, expression in stems exhibited the opposite trend, with higher values limited to the pre-stress stage. This suggests tissue-specific regulation of *SlDRO1*, with drought-induced activation in roots, potentially supporting its role in adaptive responses to water scarcity. In situ hybridization studies further localized *SlDRO1* expression in both stems and roots, providing spatial insights into its role during drought adaptation.

Ongoing efforts are focused on generating knockout (KO) and overexpression (OEX) lines of *SlDRO1* to enable full functional characterization of the gene in tomato, a first for this species. These findings will advance our understanding of the genetic determinants underlying root system plasticity and contribute to improving drought resilience in tomato and other crops.

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O-31

Identification of quantitative trait loci (QTL) underlying protein content in chickpea (*Cicer arietinum* L.) seeds

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Keywords: chickpea, seed protein content, quantitative trait loci (QTL), linkage analysis, genome-wide association study (GWAS); candidate genes

Chickpea (*Cicer arietinum L.*) is the second most important legume crop for human consumption. Being a legume, chickpea plays a crucial role in increasing agriculture sustainability by improving food security and nutrition and facilitating the transition towards plant-based diets. Indeed, its high seed protein content (17%-22%) makes the species very attractive to be used as protein source alternative to meat.

Here we characterized a wide panel of chickpea domesticated genetic resources representative of the entire geographic distribution of the species, genetically and for seed protein content, with the aim to identify genomic regions associated with protein content using a genome-wide association study (GWAS) approach.

We cultivated 202 Single Seed Descent (SSD) chickpea lines in two different field trial carried out in central Italy in two years. Seeds were analyzed for crude protein content and Whole Genome Sequencing (WGS) data were used to perform GWA.

Preliminary analyses revealed eight significant marker-trait associations (MTAs) related to protein content, distributed across chromosomes 2, 5, and 6.

These findings represent a useful tool for breeding aimed at developing chickpea varieties characterized by high nutritional value.

■

O-32

Utilization of targeted mutations to alter economically important traits in barley

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Keywords: barley, genome editing, traits

Genome editing is a revolutionary technology allowing the targeted introduction of genetic changes into the genome. We try to utilize this technology to alter various traits in barley which can potentially be useful in future crop improvements.

Width and Weight 2 (GW2) is an E3-ubiquitin ligase-encoding gene that negatively regulates the size and weight of the grain in cereal species. Therefore, disabling GW2 gene activity was suggested for enhancing crop

productivity. We showed that CRISPR/Cas-mediated mutagenesis of the barley *GW2.1* homologue results in the development of elongated grains and increased protein content. At the same time, GW2.1 loss of function induces a significant grain yield deficit caused by reduced spike numbers and low grain setting. We also show that the converse effect caused by GW2.1 absence on crop yield and protein content is largely independent of cultivation conditions. These findings indicate that the barley *GW2.1* gene is necessary for the optimization between yield and grain traits. Altogether, our data show that the loss of GW2.1 gene activity in barley is associated with pleiotropic effects negatively affecting the development of generative organs and consequently the grain production.

RNA interference (RNAi) is an essential regulatory mechanism present in the plant and animal kingdoms. RNAi is based on the sequence-specific regulation of target RNAs, and its effects are mediated by small RNAs, 20-24 nucleotides in length. In plants, different RNAi pathways play a role in the regulation of developmental processes and responses to biotic, abiotic stress factors. We are investigating the role of different protein components of the RNAi pathways in the developmental processes and molecular heat stress responses of barley by targeted knock-out of their genes. We found that components of the RNAi pathway have major role in basic developmental processes, such spike development, and also in alleviating the detrimental effects of heat stress. The generated RNAi barley mutants are associated with different phenotypes implying that individual components of the RNAi pathway can have various finely adjusted specific targets regulating diverse biological processes. These findings open the possibility to enhance economically important traits of barley by altering expression prolife of selected RNAi genes.

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O-33

Turning a green alga red: Astaxanthin biosynthetic pathway in *Chlamydomonas reinhardtii* improves resistance to light stress and biomass productivity at high irradiances

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Microalgae are photosynthetic unicellular organisms with great potential for CO_2 capture and the production of biomass and bioactive compounds. Their yield is limited by the dichotomy between the need of continuous light incoming and the dangerous effects of excess light energy that generates reactive oxygen species damaging cells. Contrasting photoinhibiton is crucial for enhancing microalgal productivity. Astaxanthin, one of the strongest antioxidants found in nature, is not naturally accumulated in many species. By engineering the astaxanthin biosynthetic pathway in *Chlamydomonas reinhardtii*, we developed a strain (bkt5) that constitutively produces astaxanthin as its primary carotenoid, dramatically shifting its color from green to red.

Engineering astaxanthin biosynthetic pathway in *Chlamydomonas reinhardtii*, we obtained a strain (bkt5) that constitutively accumulates astaxanthin as the main carotenoid, noticeably changing its color from green to red. This modification significantly increased the strain's resistance to light stress and photoinhibition, leading to improved photosynthetic efficiency and higher biomass yield. When bkt5 and its parental strain were co-cultured in extreme high light, in a "competitive test", the bkt5 outcompete the control and, within a few days, more than 90% of the cells in the bioreactor were bkt5. Changing carotenoid composition could be a viable strategy to mitigate light stress in photosynthetic organisms.

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O-34

Inositol polyphosphates regulate resilient mechanisms in the green alga *Chlamydomonas reinhardtii* to adapt to extreme nutrient conditions

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Keywords: inositol polyphosphates, *Chlamydomonas reinhardtii*, nutrient signaling

In the context of climate changing environments, microalgae can be excellent organisms to understand molecular mechanisms that activate survival strategies under stress. *Chlamydomonas reinhardtii* signalling mutants are extremely useful to decipher which strategies photosynthetic organisms use to cope with changeable environments. The mutant *vip1-1* has an altered profile of pyroinositol polyphosphates (PP-InsPs), which are signalling molecules present in all eukaryotes and have been connected to P signalling in other organisms including plants, but their implications in other nutrient signalling are still under evaluation. In this study, we conducted prolonged starvation in WT and *vip1-1 Chlamydomonas* cells. After N and P had been consumed, they showed important differences in the levels of chlorophyll, photosystem II (PSII) activity and ultrastructural morphology, including differences in the cell size and cell division. Metabolomic analysis under these conditions revealed an overall decrease in different organic compounds such as amino acids, including arginine and its precursors and tryptophan, which is considered a signalling molecule itself in plants. In addition, we observed significant differences in RNA levels of genes related to N assimilation that are under the control of the NIT2 transcription factor. These data are of important relevance in understanding the signalling role of PP-InsPs in nutrient sensing, especially regarding N, which has not directly been connected to these molecules in green organisms before. Additionally, the PP-InsPs regulation over cell size and photosynthesis supports novel strategies for the generation of resilient strains, expanding the biotechnological applications of green microalgae.

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O-35

Abiotic stress-induced chloroplast and cytosolic Ca^{2+} dynamics in the green alga *chlamydomonas reinhardtii*

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Keywords: calcium signalling, abiotic stress, microalgae

Calcium (Ca^{2+})-dependent signalling plays a well-characterized role in the perception and response mechanisms to environmental stimuli in plant cells. In the context of a constantly changing environment, it is fundamental to understand how crop yield and microalgal biomass productivity are affected by external factors. Ca^{2+} signalling is known to be important in different physiological processes in microalgae but many of these signal transduction pathways still need to be characterized. Here, compartment-specific Ca^{2+} dynamics were monitored in *Chlamydomonas reinhardtii* cells in response to environmental stressors, such as nutrient availability, osmotic stress, temperature fluctuations and carbon sensing. An *in vivo* single-cell imaging approach was adopted to directly visualize changes of Ca2+ concentrations at the level of specific subcellular compartments, using *Chlamydomonas reinhardtii* lines expressing a genetically encoded ratiometric Ca^{2+} indicator. Hyper-osmotic shock caused cytosolic and chloroplast Ca^{2+} elevations, whereas high temperature and inorganic carbon availability primarily induced Ca^{2+} transients in the chloroplast. In contrast, hypo-osmotic stress only induced Ca^{2+} elevations in the cytosol. The results herein reported show that in *Chlamydomonas* cells compartment-specific Ca^{2+} transients are closely related to specific external environmental stimuli, providing useful guidance for studying signal

transduction mechanisms exploited by microalgae to respond to specific natural conditions.

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O-36

A light touch: Protein output of duckweed cultures as a function of light intensity

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Keywords: duckweed, biomass, protein, light environment, chlorophyll meter

Due to their exceptionally rapid growth and lucky biomass composition, duckweeds (Lemnaceae) are candidate crops that produce valuable biomass for feed and biofuel. Indoor farming is one possible way of larger-scale duckweed cultivation, high operational costs due to e.g. irradiation may, however, strongly affect economic viability. Hence, optimization of culturing conditions with respect to cost-efficiency is of key importance in duckweed applications. Easy and rapid evaluation of biomass quality is another important aspect because analytical methods can be laborious and costly. Optical proxies –i.e. light absorbance- of leaves have long been used in monitoring physiological status of various crops. Analyzing light transmittance of duckweed fronds would, thus, promise quick evaluation of biomass quality. Yet, available data are still scarce on the application of such approaches on duckweeds.

We assessed how different light intensities affected growth and protein content of duckweeds. By means of a handheld chlorophyll meter, we also tested whether frond transmittance -expressed as "chlorophyll content index" (CCI)- could be applicable in estimating protein content of the biomass. A total of 11 duckweed clones were studied that covered the three rooted genera (Spirodela, Landoltia and Lemna) of Lemnaceae. The plants were cultivated under 2 different light intensities (100 and 245 µmol m^{-2} s^{-1}) in a plant growth chamber.

Higher light intensity, in general, resulted in both faster growth (average: +10%, p=0.013) and higher protein contents on area basis (average: +18%, p=0.009). The clones responded in different ways to the change in light environment: the plant area-based protein productivity increment due to higher light ranged between -2 and +36% (+25% as an average, p=0.002). This gain, however, came at an expense of ~150% higher energy consumption. Our results, thus, point to the importance of jointly addressing both ecological and economic constraints. CCI of fronds proved to correlate well with protein contents. Optical proxies, therefore, can be assumed as reliable and quick tools in assessing duckweed biomass quality in larger-scale applications too.

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O-37

Modeling of microalgal growth and biochemical composition under nitrogen fluctuations

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Keywords: microalgae, mathematical modeling, nitrogen starvation, lipid accumulation, bioactive compounds, biomass valorization

Microalgae are promising sources of high-value biomolecules due to their ability to accumulate lipids, sugars, and proteins in response to nutrient availability. This study presents an extended mathematical model of microalgal growth and macromolecule accumulation–neutral lipids, starch, and proteins–under both nitrogen-replete and nitrogen-limited conditions: N limitation and N starvation. Building on a previous framework, three new equations describe lipid, sugar, and protein production, based on biomass concentration and specific N uptake rate and quotas, when nitrogen is available. Under sudden nitrogen starvation, cell division and protein synthesis stop, but biomass dry weight increases through lipid and starch accumulation, driven by

starvation-specific rates. The model captures these shifts in biomass composition with newly defined dynamics.

As a complementary line of research, microalgal extracts are being analyzed for their bioactivity against fungal infections in plants. Preliminary investigations aim to isolate and identify novel bioactive compounds capable of eliciting plant defense responses, paving the way for sustainable plant protection strategies.

The integrated modeling and experimental approach provide a comprehensive understanding of microalgal metabolic responses to nutrient availability, offering valuable insights for optimizing biomass valorization and developing bio-based solutions for agriculture.

O-38

Inter-kingdom interactions within natural and synthetic algal bacterial communities

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Past empirical research has demonstrated that bacterial interaction might enhance algal biomass production and modify biomolecule composition (including algal EPS and lipid production as well as biohydrogen evolution patterns). To investigate the mechanisms and microalgal functions activated under bacterial associations different bacterial species were co-cultivated with various eukaryotic green microalgae, including *Chlamydomonas reinhardtii* cc124 green algae. Bacterial species were isolated from diverse environments including biogas sludge, soil and commercial plant biostimulant products. Pairwise algal-bacterial combinations were cultivated for five days in synthetic wastewater. The accumulated biohydrogen was recorded, the specific algal growth rate was determined, co-cultivation specific physiological and morphological alterations were investigated. Successful bacterial candidates were identified by high algal biohydrogen production as well as by increased algal biomass and lipid production. The effects of bacterial phylogenetic relationship and growth rate on algal functions such as biomass yield, nutrient uptake and biomolecule composition were analyzed. The mechanisms of the interactions were investigated using transcriptome analysis and advanced microscopy techniques.

O-39

Interspecific hybridis and polyploids in duckweed

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Keywords: duckweed, hybridization, polyploidy

Members of the small family of free-floating, aquatic macrophytes Lemnacea are cosmopolitan inhabitants of freshwater bodies. Under the name of duckweed or water lentils, they are attracting increasing interest due to the exploitability of their fast growing biomass for wastewater remediation, as well as for feed and food production. In particular, four different species belonging to the genera *Wolffia* and *Lemna* have been approved in 2021 and this year, respectively, as novel food in the EU. In addition, easy of cultivation, fast generation time, amenability for genetic transformation and possibility of flower induction, make duckweed a suitable model plant for a number of fundamental studies.

The rising popularity of duckweed calls for a deeper knowledge of this monocot family, and a better understanding of its genetic diversity and evolutionary history. Taxonomy and species delimitation have been hindered since long by the intrinsic difficulty posed by the extremely simplified water body of most species, with few differentiated structures and tissues.

We set up a combination of approaches integrating plastid and nuclear molecular markers, flow-cytometric genome size measurements, chromosome counting, genomic *in situ* hybridization (GISH) and whole-genome sequencing (WGS), to increase our knowledge in the field.

We discovered that, despite predominant asexual propagation, the Lemnacea did not completely abandon sexual reproduction, including the possibility of breeding with closely related species. Outcrossing produces a network of previously overlooked species complexes, including interspecific hybrids of variable ploidy in the genus *Lemna*, providing high genetic diversity.

The evolutionary potential of different hybrid lineages is explored through the investigation of sexual reproduction in these plants, shedding light on possible evolutionary pathways and future trajectories.

Studies on the genetic diversity of the *Wolffia* and *Wolffiella* genera are also ongoing, leveraging a large duckweed germplasm collection available at our Institute since 2021. First evidence suggests that hybridization and triploidy are as common as in the genus *Lemna*.

This work was supported by Italian CNR within the Agritech National Research Center and received funding from the European Union Next Generation EU [grant ID PNRR, Missione 4 Componente 2, Investimento 1.4—Project CN00000022].

O-41

Exploiting tailored microbial consortia to enhance sustainability and resilience of grapevine cultivation

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Keywords: arbuscular mycorrhizal symbiosis, grapevine, root-associated microorganisms

Besides plant genotype, environmental factors such as nutrient and water availability, as well as the ability of the plant to properly utilize what is available in the soil, play a relevant role in plant growth and productivity. Crop and soil microbiota diversity are pivotal for addressing environmental threats, improving crop yields, and promoting soil health. The Micro4Life project is focused on grapevine with the aim to i) define and provide traits involved in the interactions with soil microorganisms; ii) verify the influence of natural growth environments and soil conditions on the interactions with soil microorganisms and *vice versa*; iii) actively engage with relevant stakeholders and disseminate new knowledge on the use of root-associated microbes and on traits relevant to develop resilient crops to environmental stresses. Micro4Life will generate new information on root traits relevant to resource use efficiency (both in terms of exploitation and exploration) and tolerance to stresses, including the capacity to recruit beneficial soil microorganisms. Particularly, for the latter aim, ten different rootstock genotypes have been evaluated for their specific responses (microbiome, transcriptome and metabolome) to the inoculation with a custom-designed microbial consortia. In parallel, commercial and newly developed arbuscular mycorrhizal fungi (AMF) inocula were tested in greenhouse on different rootstock (*Vitis* spp.) genotypes. Beside colonization efficiency, effects of the applied AMF inocula on morphometric and physiological features were studied, paying attention to specific genotype x AMF inoculum interaction. Consortia of selected beneficial microbes to boost grape resilience to climate change will be developed, also based on physiological and agronomic data collected over two consecutive seasons in open fields.

This work was supported by the Micro4Life project (Ager3 Call, grant n° 2022-2903).

O-42

Growth and secondary metabolite modulation by soil beneficial micro-organisms in *Artemisia annua* plants

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Keywords: artemisia, soil beneficial microorganisms, volatilome

The family Asteraceae includes many genera among which *Artemisia* is one of the most widespread in the world. *Artemisia annua*, a herbaceous annual plant commonly named "annual absinthe", produces a high number of secondary metabolites among which VOCs (Volatile Organic Compounds) and artemisinin, a sesquiterpene lactone with antimalarial action. Since the inoculation with soil beneficial microorganisms, such as arbuscular mycorrhizal fungi (AMF) and plant growth promoting bacteria (PGPB), is one of the strategies used to improve plant growth and metabolism, in this work

different AMF and PGPB inocula were tested, alone or in combination, on two clones of *A. annua* (CL7 and CL26) grown in controlled conditions. Plants were irrigated three times a week with a Long Ashton nutrient solution at 32µM of P and harvested after two months of culture. Then, the mycorrhizal colonization (M%) in the plant root system, the shoot and root fresh and dry weight, the leaf chlorophyll and carotenoid concentrations and the quantitative (HPLC) analysis of artemisinin were evaluated. In addition, the composition of the leaf volatile profile of CL26 plants was analyzed by GC-MS. Data were statistically analyzed by two-way ANOVA using "fungus" (F) and "bacterium" (B) as factors. A one-way ANOVA followed by Fisher test with significance cut-off at $p<0.05$ was used to assess differences among the treatments. Results showed that the various bacterial and/or fungal inocula had different impact on growth and metabolism of the two different clones. Some bacterial strains were found to increase the ability of AMF to colonize the plant roots, even if the M% remained rather low. In general, no significant differences for the artemisinin content were recorded between the different treatments of each clone, however the same bacterial strain (Pf7) led to a decrease content of artemisinin in CL7 clone, while it increased its production in CL26. Regarding the volatile profile, 120 molecules (some of which detected for the first time in this plant species) were identified. The volatilome composition varied according to the different inocula since some molecules were exclusive of a certain treatment and others showed variations in their proportions. Therefore, plants of each treatment had their own specific particular odor due to the interaction between plant and soil beneficial microorganisms.

O-43

Cork endophytic microbiome: Characterization and impact on plant development

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Keywords: cork oak, metagenomics, phellem, phellogen, root development

Cork oak (*Quercus suber* L.) is a symbolic species of the West Mediterranean region, known for the production of cork. With its wide number of applications, cork is a very valuable material. As such, there has been a growing interest in finding ways to enhance growth and mitigate the effects of severe drought. The novel production practices that have been proposed have been based on the use of irrigation and fertilization. Nevertheless, the improper use of irrigation and fertilization may lead to detrimental effects on the environment. To attempt to reduce water and fertilizers requirements, complementary approaches may be developed through the exploration of the potential of beneficial plant-associated bacteria, such as endophytes with growth-promoting effects. The present study aimed to (i) identify endophytic communities present in living cork tissue, (ii) characterize the diversity occurring in trees producing cork with contrasting thickness, and (iii) assess the impact of the cork-associated bacteria on plant development. A metagenomics analysis was performed on cork oak bark phellem/phellogen samples collected from trees producing thicker (Top Plus) and thinner (Top Minus) cork planks. The Top Plus group exhibited a distinct composition in comparison to Top Minus. While in samples from thicker cork planks *Pseudomonas* and *Sphingomonas* were the most abundant genera, in thinner cork planks *Acinetobacter* was the most abundant genus. Furthermore, culturable bacteria were isolated from bark phellem/phellogen and their impact on plant development was evaluated. To this end, *A. thaliana* roots were used as a model, with most isolates showing an impact on root architecture. The molecular mechanisms regulating such changes are currently being targeted, and the most promising bacterial isolates have been selected for further studies in cork oak saplings.

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O-44

Unveiling the hidden allies of industrial chicory: A metagenomic exploration of rhizospheric microbiota and their impact on productivity and plant health

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Keywords: chicory, rhizosphere, microbiome, metagenomics

As industrial chicory holds significance for food, fodder, and medicinal purposes, its cultivation is increasingly crucial for producers. To enhance productivity, resistance, and the nutritional and functional values of this plant, we aimed to investigate its interactions with the microbial environment. We conducted the first comprehensive taxonomic and functional characterization of the rhizosphere microbiota associated with industrial chicory, examining how environmental factors influence its composition. Six different land plots were simultaneously cultivated with the same chicory genotype in the North of France. Using soil analyses and metagenomic approaches, we characterized the diversity of bacterial and fungal communities in the soil microbiome associated with chicory plants and discussed their functional traits.

We observed significant taxonomic variability, influenced by substrate composition and cultivation history across each plot. The presence of chicory plants distinctly shaped the microbial community. Specifically, chicory was found to recruit *Streptomyces* species that produce plant hormones, as well as *Penicillium* species that facilitate phosphate solubilization and promote plant growth. Moreover, the plant demonstrated an ability to repel pathogens and adapt to local microbial communities by selectively favoring beneficial microorganisms according to local stresses and nutritional needs. Our study represents the first comprehensive taxonomic and functional analysis of the *Cichorium intybus* rhizosphere microbiome, underscoring the pivotal roles of soil composition and land use history. The specific microbial recruitment by chicory was also discussed.

O-45

Serendipita indica promotes *Arabidopsis thaliana* growth through nuanced modulation of auxin homeostasis and transport

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Keywords: auxin homeostasis, growth promotion, endosymbiosis

Agricultural productivity is increasingly jeopardized by the ongoing climate change scenario, calling for more holistic and environmentally sustainable strategies to enhance plant productivity and ensure food security. In this context, root-colonizing fungal endophytes have been demonstrated to significantly enhance plant growth and resilience under both biotic and abiotic stress conditions. Our research aimed to elucidate the molecular mechanisms by which the beneficial root-colonizing endophytic fungus, *Serendipita indica*, promotes growth in *Arabidopsis thaliana*. Utilizing a comprehensive methodology that includes transcriptomics, metabolomics, and reverse genetics, it was demonstrated that *S. indica* modulates auxin distribution in *A. thaliana* roots by influencing auxin transport and conjugation. Specifically, the fungus suppresses the expression of the auxin transporter gene *PIN2*, resulting in auxin accumulation at root tips and the subsequent activation of *GH3* genes. The study identified GH3.5 and GH3.17 as critical regulators of biologically active free auxin levels within the examined plant-fungus interaction. This intricate modulation of auxin homeostasis is crucial for the symbiotic relationship and the resultant increase in plant biomass. The findings provide novel insights into plant-microbe interactions and underscore the significant role of auxin in plant-fungus interactions, suggesting a potential pathway for enhancing agricultural productivity in the context of climate change.

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O-46

Enhancing tomato plants' resilience to climate change: The role of mycorrhization and strigolactones

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Keywords: arbuscular mycorrhizal fungi, metagenomics, photosynthesis, salt stress

Currently, tomato production within the Mediterranean basin faces great challenges due to climate change, namely the growing incidence of heat peaks and soil salinity. In this context, efforts to tackle these issues are particularly relevant. Thus, this research aimed at understanding if arbuscular mycorrhizal fungi (AMF; root inoculation with a commercial AMF inoculum – Symbivit®) and/or strigolactones (SLs; foliar spray with 5 µM GR24, a synthetic analogue) could improve tomato plants' resilience to a hot (>35 ºC for most of the pre-harvest period) and saline (three-week irrigation with 100 mM NaCl) environment. The experimental trial lasted between April and August 2023, covering the plants' life cycle from germination to fruit maturity. Briefly, data showed salt-irrigated plants presented reduced photochemical efficiency, whereas this effect was not observed upon treatment with AMF or SLs. However, regarding productivity-related endpoints, plants responded differently to the treatments. Here, plants treated with AMF exhibited significant improvements in growth, fruit production, and overall yield, even under stressful conditions, correlating with an enhanced gas exchange ability. On the other hand, although SLs are usually associated with stress tolerance and the promotion of mycorrhization, their application did not provide additional benefits beyond those conferred by AMF alone, actually negating some of these effects. Furthermore, the applied treatments and saline conditions influenced fruit composition, altering attributes such as sugar content and phenolic acid levels. Similar differential modulations were also observed in microbiome composition, emphasizing the complex interplay between plant physiology and soil microbial communities. These findings underscore the importance of a holistic, multi-level approach when assessing plant stress responses in agricultural contexts and highlight AMF inoculation as a viable and sustainable strategy to enhance tomato resilience against heat and salinity, ensuring stable yields and maintaining fruit quality under realistic conditions.

O-47

Symbiotic NCR-like peptides and bacteroid differentiation in *Amorpha fruticosa - Mesorhizobium amorphae* symbiosis

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Keywords: biological nitrogen fixation, NCR peptides, *Amorpha fruticosa*

Nitrogen is an essential element for all living organisms, yet atmospheric N_2 can only be utilized by certain bacteria and archaea that convert it to ammonia via the nitrogenase enzyme complex. The most efficient biological nitrogen fixation occurs in the symbiosis between rhizobium soil bacteria and leguminous plants, where bacterial cells differentiate into nitrogen-fixing bacteroids within root nodules. In legumes of the Inverted Repeat-Lacking Clade (IRLC), this differentiation is regulated by host-derived peptides, including nodule-specific cysteine-rich (NCR) and glycine-rich (GRP) peptides, which induce irreversible terminal differentiation of bacteroids. *Medicago truncatula* harbors over 700 NCR peptides, leading to the formation of enlarged, polyploid, uncultivable bacteroids with enhanced nitrogen fixation efficiency.

Terminal bacteroid differentiation has also evolved in Dalbergoid legumes, alongside the emergence of NCR-like peptides. *Amorpha fruticosa*, a widely distributed invasive leguminous shrub, forms symbiosis with its native rhizobial partner, *Mesorhizobium amorphae*. Despite its ecological significance, little is known about the infection process, symbiotic peptides, or bacteroid differentiation in this system. Our research aims to uncover the molecular mechanisms of symbiosis in *A. fruticosa* by identifying symbiotic NCR-like peptides, characterizing bacteroid differentiation, and analyzing the nodule transcriptome and genome. We will present new insights into the infection process, symbiotic peptide repertoire, and nitrogen fixation potential of this invasive perennial shrub, contributing to a deeper understanding of legume-rhizobium interactions beyond the IRLC.

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O-48

Responses of maize roots and the rhizosphere microbiome to alternating precipitation: Insights from a three-year field study

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Keywords: ACC deaminase, monoculture, superoxide dismutase

Addressing crop production challenges caused by annual weather variability, including droughts, requires an improved understanding of plant-soil-microbe interactions. Drought-induced changes in root growth and exudation allow plants to influence the rhizosphere microbiome and enzyme activities, which can enhance their drought tolerance. To better understand how these feedback processes are affected by drought, root gene expression, rhizosphere microbiome community composition and rhizosphere enzyme kinetics of maize plants, grown on the substrates sand and loam, were investigated during two dry and one moist year for the maize wild-type (WT) and respective roothairless 3 (*rth3*) mutant. We expected dry years to stimulate a drought stress related root gene expression pattern, lower rhizosphere enzyme activities, but higher relative abundancies of gram-positive bacteria. We furthermore expected a substrate effect that is more pronounced in dry years. Both dry years showed a drought-related gene expression pattern compared to the moist year. Interestingly, more differentially expressed genes encoding dehydrins and heat shock proteins were found between the second dry year and the moist year than between the first dry year and the moist year. This suggests that low rainfall in previous years may have had a legacy effect on the plant's response to drought. Increasing levels of root stress enzyme activities in sand over the years furthermore indicate an effect of repeated maize planting. Surprisingly, we observed higher maximum enzymatic rates in the rhizosphere in dry years. Together with increased levels of gram-positive bacteria under drought, this may indicate an adaptation of the rhizosphere microbiome to drought conditions. The substrate effect was found to be more pronounced during dry years only for root gene expression levels, in contrast to the rhizosphere microbiome. Our results reveal interactive effects between maize roots and the rhizosphere microbiome and how they respond to dry and warm climate, presumably influenced by earlier dry periods and repeated maize planting. To disentangle these interactions, future research directions should further address plant-soil-microbe interactions in long-term field experiments, facing not only changing environmental conditions, but also soil legacy effects.

O-49

Phaseolin, a new eco-friendly protein-based bioplastic

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Keywords: biopolymer, disulfide bridges, phaseolin

Petroleum-based materials have acquired more importance in our lives. However, the wide use of plastic materials has proved to be very problematic for the ecosystem representing today one of the major sources of pollution. Therefore, the scientific community is constantly looking for strategies to produce eco-friendly bioplastics that can replace petroleum-based materials, and this present work proposes a strategy that could partially replace them.

We chose phaseolin, an edible protein that represents the 50% of the vacuole protein content in common bean seeds. In its native form, phaseolin organizes in homotrimers but is unable to form longer polymers. Genetic modification of its coding sequence introduced a codon for cysteine at the C-terminal tail of the protein (named PHSL*) that leads to the formation of disulfide bridges between the phaseolin units, resulting in the production of a long polymeric chain of the protein[1]. We employed the biolistic transformation technology to introduce PHSL* gene into the plastidial genome of *Nicotiana tabacum* plants and the transformation of the chloroplasts was chosen because its oxidizing environment promote the correct production of the disulfide bridges; once the biopolymer is produced it can be used to make several products[2].

Plasticization tests revealed the formation of thin films of biopolymer, which are transparent, insoluble and elastic. We further modified the engineered phaseolin structure by inserting additional cys, in order to produce a biopolymer that could be even bigger and stronger. This new construct is being used to transform crops to take advantage of use the large amounts of waste they generate to produce bioplastics. In a circular economy model where waste becomes a resource future application of this new biopolymer in different sectors, ranging from packaging for food industry to biomedical sector as a carrier of protein-based drugs.

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O-50

Metabolite repair in plants: A nudix enzyme's role in thiamine salvage

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Keywords: metabolite repair, plant metabolism, vitamin B_1 metabolism

Metabolite damage and repair concepts are new and at the frontier of metabolic research. Metabolite damage arises because enzymes in all organisms, although regarded as specific, frequently make mistakes that lead to toxic damage products. Such products can also arise from spontaneous chemical side reactions of metabolites. Genetic and genomic evidence from prokaryotes and eukaryotes has implicated a network of conserved enzymes that repair damaged metabolites. Understanding damage and repair processes is foundational to interpreting and manipulating metabolism, and knowledge of such systems is crucial for successful biotechnology applications.

A prime example of metabolite damage and repair occurs in thiamine (vitamin B_1) metabolism. Thiamine is essential for properly functioning many central metabolic enzymes. While plants and most bacteria can synthesize thiamine, animals must acquire it from their diet. Thiamine biosynthesis is well understood in both bacteria and plants. In bacteria, thiamine is phosphorylated by thiamin kinase (ThiK) and monophosphate kinase (ThiL). In contrast, in plants, thiamine monophosphate is first dephosphorylated (TH2) and then pyrophosphorylated by a pyrophosphate kinase (ThDPK). However, like many other metabolites, thiamine is susceptible to damage, forming compounds such as oxothiamine and oxythiamine. The enzyme responsible for thiamine pyrophosphorylation, ThDPK, can also phosphorylate these damaged forms. Previously, it was thought that plants lacked a dedicated salvage enzyme to repair damaged thiamine derivatives. However, we have identified a Nudix enzyme capable of selectively dephosphorylating these damaged thiamine forms, thereby preventing their accumulation. Comparative genomics evidence further suggests that the TenA domain of the TH2 gene may also contribute to salvaging oxothiamine and oxythiamine. This TenA domain belongs to the TenA_C subfamily, whose other members exhibit amino-HMP aminohydrolase activity, highlighting the evolutionary adaptation of metabolite repair mechanisms in plants.

O-51

Carnivorous sundew is a rich source of hydrolases: A story of a peculiar protease

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Keywords: *Drosera binata*, gene expression, hydrolytic enzymes, molecular cloning

Carnivorous plants are a diverse group of flowering plants that grow in nutrient-poor and wet habitats. To compensate for limited macroelements, they attract arthropods and capture prey using specialized trap leaves, subsequently triggering the expression of specific genes coding for digestive enzymes. Our understanding of transcriptomic changes upon insect capture and the tissue-specific expression of hydrolytic enzymes is still fragmentary and needs deeper investigation. Furthermore, proteins discovered in the trap secretions are a valuable source of hydrolases for various biotechnological applications. Therefore, the aim of this study was to acquire the complete gene sequence of a candidate cysteine protease, previously identified through proteomic profiling of induced trap leaves from carnivorous sundew, and evaluate its transcription dynamics in different plant organs. The forked-leaved sundew, *Drosera binata*, was aseptically cultivated on supplemented 25% Murashige and Skoog medium. Nucleic acids were extracted from plants with high biomass using an optimized CTAB protocol. We performed the genome walking method to acquire the full-length genomic region of the sundew hydrolase. The resulting protease gene sequence, which is 1684 bp long with an adjacent promoter region of 516 bp, was subjected to comprehensive *in silico* analysis employing available bioinformatic tools. The open reading frame of 1038 nucleotides was predicted to encode a polypeptide of 346 amino acids, and comparison of the genomic and cDNA sequences revealed the presence of one intron. First-strand cDNA from the root, stem, flower, and sequential developmental stages of trap leaves were subjected to probe-based quantitative real-time PCR with *de novo*-designed gene-specific primers to elucidate the transcriptional profile of the selected *D. binata* protease. The obtained data were normalized to the expression level of the endogenous reference gene and analyzed using the $2^{-\Delta\Delta Ct}$ method. Exciting results regarding the protease gene expression dynamics in sundew will be presented at the conference. In conclusion, we isolated a full-length protease-coding gene sequence involved in prey digestion that had not been previously described in sundew, providing a promising candidate for further recombinant protein expression and characterization.

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O-52

G-protein alpha subunit negatively regulates nitrogen use efficiency in rice

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Keywords: G-protein, nitrogen-use-efficiency, rice

Crop Nitrogen Use Efficiency (NUE) improvement is crucial to prevent economic wastage of expensive N-fertilizers and to prevent their environmental impacts such as air/water pollution, illhealth, biodiversity loss and climate change. Rice is an ideal target for improvement of NUE in India due to its highest urea consumption and huge genetic and genomic resources. We characterized the phenotype for NUE and used transcriptomics to identify the underlying traits/genes/processes in Indica and Japonica subspecies of rice (eg: Mandal et al., 2022). Our earlier transcriptomic analysis of G-protein α subunit mutant in Japonica rice (d1 mutant) revealed many genes/processes common with nitrate response (Pathak et al., 2021). Recent pot studies in the greenhouse revealed that the Gα mutant had lesser N-dose sensitivity, higher grain yield and NUE than WT, revealing that G-protein negatively regulates NUE through N-dose sensitivity. Physiological evaluation of the Gα mutant revealed that its high NUE was associated with better photosynthetic performance, transpiration efficiency and internal water-use efficiency in comparison to WT (Prasanna et al., 2023). Further, the altered genome-wide nitrate-response in the Gα mutant transcriptome relative to the WT revealed many candidate genes that are both nitrate-responsive and G-protein regulated. Attempts are currently underway to generate genome-edited knockouts for G-protein subunits and other candidate genes for NUE in popular Indica rice genotypes to improve NUE.

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Chloroplast-nucleus communication: Protein trafficking for plant immunity

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Keywords: organelle, interorganellar communication, plant immunity

Organelle communication within a plant cell is essential for effectively exchanging signals that regulate acclimation under stress conditions. Chloroplast is a major site for producing defense-related molecules and communicating with other organelles during immune responses. Rapid communication between the chloroplast and nucleus is vital for an effective immune response. While little is known about the transfer of molecules from chloroplasts to the nucleus during these responses, emerging evidence indicates that signaling molecules, including proteins and metabolic products, are transferred from the chloroplast to the nucleus. Here, we investigate the translocation of proteins from the chloroplast to the nucleus during immune responses. Through comparative nuclear proteomics under active immunity and normal growth conditions, we identified 195 putative translocating proteins enriched during the immune response, primarily involved in various cellular and metabolic processes. For example, the chloroplast-localized protein NADPH-dependent thioredoxin reductase C (NTRC) is translocated to the nucleus via stromules under the immune response. Furthermore, in vitro interaction studies using the high-throughput yeast two-hybrid assay reveal that NTRC interacts with several transcription factors. We further confirmed these interactions in plant cells, suggesting the NTRC might be involved in transcriptional reprogramming in plant immunity. Uncovering the new roles of these candidate proteins will enhance our understanding of the molecular mechanisms driving inter-organelle communication during plant immune response.

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From integrity to division: How cell wall status controls the cell cycle in plants

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Keywords: Cell Wall Integrity (CWI), mechanochemical signaling, cell cycle regulation

The plant cell wall is more than a structural scaffold–it is a dynamic regulator of growth and development. However, how plants sense their cell walls and adjust cell cycle activity remains unclear. In this talk, I will present evidence for a mechanochemical feedback loop linking cell wall composition, stiffness, and cell cycle progression in *Arabidopsis thaliana*. We used cell cycle mutants from key regulatory families–including cyclins, DEL transcription factors, KIP-related proteins, E2F transcription factors, and cyclin-dependent kinases (CDKs)–alongside targeted chemical treatments affecting pectin, cellulose, or both. This allowed us to examine how modifications in cell wall composition influence the cell cycle, and vice versa. Monosaccharide profiling revealed altered wall composition in these mutants, particularly altered levels of galactose, glucose, and glucuronic acid. Based on these findings, we selected representative mutants for mechanical analysis using Brillouin microscopy. Preliminary results suggest that stiffer cell walls correlate with delayed cell cycle progression, while softer walls promote endoreplication, indicating that mechanical properties actively regulate cell cycle transitions. We also identified the receptor-like kinase THESEUS1 (THE1) as a central regulator required to couple wall signals to cell cycle control. Together, these findings show that plants actively integrate mechanochemical cues into cell cycle regulation, linking cell wall integrity to growth control. This has implications for stress resilience, biomass optimization, and crop improvement.

O-55

The impact of cell shape on cellular microviscosity dynamics in response to abiotic stress-induced-cell wall integrity impairment

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Keywords: cellular microviscosity, cell wall integrity, abiotic stress

The plant cell wall plays a pivotal role in enhancing crop resilience, as it serves as the primary barrier against environmental stress. Unfortunately, manipulating cell wall structure and metabolism remains challenging due to its plasticity. Cell wall plasticity refers to the wall's ability to alter its shape, composition, and viscoelasticity (stiffness and viscosity) in a highly controlled manner in response to wall alteration caused by stressors. However, any impairment in cell wall functionality seems to be detected by the cell wall integrity (CWI) maintenance mechanism. It is the key mechanism that monitors the functional integrity of cell walls by utilizing a wide range of receptor-like kinases and ion channels in the plasma membrane to detect cell wall impairment and translate it into signals initiating wall remodeling. One of the cellular properties affected by this signaling is microviscosity, which influences the diffusion rates of all molecules and is crucial for proper cellular function. This project aims to investigate the relationship between cell shape and cell microviscosity during CWI impairment caused by exposure to abiotic stress by using fluorescence molecular rotors. A molecular rotor is a fluorescent probe that undergoes internal rotation upon photoexcitation, with the rotation rate directly reflecting the viscosity of its surrounding environment. The goal of this project is to construct a highly detailed microviscosity map of cells and tissues at a subcellular resolution. This approach will provide invaluable insights into cellular dynamics, revealing how systems respond to various forms of abiotic stress and highlighting the critical role of CWI maintenance. Such knowledge is not only essential for understanding plant resilience but also for developing strategies to improve crop performance in the face of ever-increasing environmental challenges.

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Unravelling transcriptional changes at single cell resolution at early stages of Root Knot nematode infection

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Keywords: scRNAseq, RKN, nematode cluster

Root Knot nematodes (RKN) are plant parasitic nematodes (PPNs) responsible of worldwide agricultural losses affecting all major crops. RKN infect plant roots and hijack developmental programs to form a pseudo-organ called the gall. During this process of infection, root cell types respond differently to the nematode as cortex cells hypertrophy, vascular cells proliferate, and a few cells (5-8) differentiate into giant cells (GCs). To unravel the cell-type specific transcriptional responses to nematode infection, we performed a single cell RNA sequencing (scRNAseq). Due to the complexity of our biological system and scRNAseq technique, we implemented infection protocol and tracked the nematode infection to select the most suitable time to capture the desired gall developmental stages compatible with protoplast isolation. Then, we performed scRNAseq on three biological replicates from mock and *Meloidogyne javanica* inoculated roots of *Arabidopsis thaliana.* A high-resolution dataset was obtained containing 35594 and 14153 high quality cells and a median of 1970 and 2005 genes per cell in the mock and inoculated samples respectively. An unsupervised clustering analysis identified 28 major clusters, with two distinct clusters enriched in infected cells forming what we called "nematode clusters". The "nematode clusters" is formed by a heterogeneous cell population of responding cells during RKN infection including undifferentiated quiescent stem-like cells only found in the nematode sample, and not in the mock sample. This analysis suggests major plant cell reprogramming and acquisition of a pluripotent cell stage during the early

stages of the nematode infection. Furthermore, looking at differentially expressed genes on the specific quiescent stem-like cells only present in the nematode sample followed by functional studies, we were able to identify two putative key gene players during the early stages of nematode gall formation.

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The roles of PP2A subunits Fass and C3/C4 in the regulation of mitosis and oxidative stress responses in Arabidopsis- studies with phosphatase mutants and inhibitors

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Keywords: protein phosphatase PP2A, Fass subunit, C3-C4 subunits, phosphohistone H3, mitosis, oxidative stress

PP2A, a family of serine-threonine protein phosphatases is a complex consisting of A (scaffolding), B (regulatory) and C (catalytic) subunits. By using wild-type and loss-of-function phosphatase-related mutants (*fass* for a B" and *c3c4* for two catalytic subunit isoforms that interact with Fass in wild-type plants) of Arabidopsis, we have found that as expected, C3/C4 subunits are responsible for a significant proportion of total PP2A activity, and surprisingly affect the activity of a related phosphatase, PP1 as well. We show for the first time that *fass* mutants are not impaired significantly in total phosphatase activity, because instead of decreasing PP2A, the balance between PP2A-PP1 activities is altered. Immunohistochemical studies for microtubules and phospho-histone H3 show that both types of PP2A subunits regulate mitotic activity in the root apical meristem and influence the phosphorylation of histone H3 during mitosis, a process important in the regulation of metaphase-anaphase transition and the entire mitotic process. Fass exerts this function not only by regulating PP2A (possibly involving its subcellular localization), but also by influencing PP1 activity. The two types of subunits are affecting reactive oxygen species (ROS) production (histochemical studies) and scavenging as well. By studying the activities of different superoxide dismutase (SOD) isoforms, we found that the primary target of Fass and in relation to this, C3 and C4 is Cu/Zn SOD. The protein phosphatase inhibitor microcystin-LR increased the susceptibility of mutants to both mitotic anomalies and alterations of ROS metabolism.

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cAMP – A tale of two messengers

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Keywords: cAMP, channel, net ion fluxes

Cyclic mononucleotides, including 3',5'-cyclic adenosine monophosphate (3',5'-cAMP) and its positional isomer 2',3'-cyclic adenosine monophosphate (2',3'-cAMP), are increasingly recognized as key signaling molecules in plants. 3',5'-cAMP is synthesized from ATP by adenylate cyclases (ACs) and metabolized by cyclic mononucleotide-specific phosphodiesterases (PDEs). On the other hand, 2',3'-cAMP is an RNA degradation product that accumulates under stress and may play a role in stress signaling, although its specific mechanisms of action remain unclear.

This study aimed to investigate the differences between the two isomers, both systemically and locally.

Proteomic analysis showed that, despite some overlap, the two isomers affect distinct sets of proteins, suggesting that they play diffferent roles in cellular processes.

We further investigated the specific effects of these two isomers on ion fluxes. MIFE (non-invasive Microelectrode Ion Flux Estimation) analysis revealed that both isoforms contribute to the reduction of K+ net ion fluxes in roots during oxidative stress, supporting their role in stress protection, with some differences in their relative impact. .

Finally, to determine whether 2′,3′-cAMP and 3′,5′-cAMP share the same targets in signal transduction via cyclic nucleotide-gated channels (CNGCs), Two-Electrode Voltage Clamp (TEVC) experiments were performed. The *Arabidopsis thaliana* CNGC2 channel (AtCNGC2) was selected as a target because of its annotated role in the abiotic stress response. Experiments using *Xenopus laevis* oocytes expressing AtCNGC2 revealed that while 3′,5′-cAMP induced K^+ currents, whereas 2′,3′-cAMP failed to elicit any currents, indicating no specific interaction with the channel.

Taken together, these results provide experimental evidence that the two cAMP isomers modulate distinct pathways, reflecting differences in their molecular targets.

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O-59

Homogalacturonan pectins as an element of rbohD/F and rbohD response to TuMV infection

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Keywords: cell wall, plant viruses, plant-innate immunity, resistance

The plant cell wall is integral, valid and active element of response against various biotic stresses (like bacteria or fungi). Bacterial and fungal pathogens has different methods to actively move through structure of the cell wall, but one of the types of pathogens, viruses are not able to mechanically destruction of host cell walls. This fact created unique gap in understanding cell wall remodeling in response to plant viruses. To investigate this scientific problem *Turnip mosaic virus* (TuMV) and *A.thaliana* mutants with different type of response against this virus (*rbohD/F* -hypersensitive like reaction /incompatible, *rbohD*-increased susceptibility /compatible) pectin role were explored. We selected pectins as one of the most valid elements of the primary cell wall which are also frequently engaged in response to pathogens. The HGs (homogalacturonans), a main component of pectins, have been known as defensive molecules directly associated with resistance responses. Regardless of the interaction type, our ultrastructural results indicated cell wall remodeling process as active part of response to TuMV. In the compatible reaction promoted in *rbohD*-TuMV interaction, there was upregulation of *AtPME3* (pectin methylesterase), but not *AtPME17*, confirmed also PME3 protein deposition. Moreover, the highest PME activity along with a decrease in cell wall methylesters compared to resistance interactions in *rbohD/F*–TuMV were noticed. These findings suggest that selected PME enzymes have a diverse impact on the demethylesterification of HGs and metabolism as a result of *rboh*–TuMV interactions, and are important factors in regulating cell wall changes depending on the type of interaction, especially in resistance responses. Therefore, the role of pectins should be investigated further for better understanding of its role during plant-viral interactions.

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O-60

The chloroplast located HKT transporter plays an important role in fertilization and development in *Physcomitrium patens*

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Keywords: HKT, Physcomitrium, thylakoid

Ion transport across biological membranes plays an important function in maintaining the ionic homeostasis within the organelles for the well-functioning of a cell. Any alteration in these processes will reflect in the malfunctioning of any organelle, and consequently, in the development of the organism. In plant cells, sodium transporters play a central role in keeping the concentrations of this cation across all membranes under physiological conditions to prevent its toxic effects. HKT transporters are a family of membrane proteins exclusively present in plants, with some homologs present in prokaryotes. HKT transporters have been associated to salt tolerance in plants, retrieving any leak of the cation into the xylem, or removing it from aerial parts, including the flowers, to be transported to the roots along the phloem. In this work we identified the

sole HKT transporter (*Pp*HKT) present in the moss *Physcomitrium patens*. The predicted tridimensional protein structure presents four Glycines in the pore region of the selection filter, similar to the related Trk/Ktr prokaryote K^+-selective transporters. We identified the localization of *Pp*HKT3XmNeon to the thylakoid membrane and its functioning as a Na^+/K^+ co-transporter in yeast. The phenotype for the *PpΔhkt* mutant causes several defects in gametophore growth, a lower chlorophyll content and defects in the fertilization process causing a null production of sporophytes, suggesting an essential role in the development and metabolism of the moss. In addition, RNA-seq analysis of the mutant reveals the down expression of genes involved in photosynthesis and spermatogenesis. In contrast, genes involved in ion and metal membrane transport are up regulated. These results suggest the importance of HKT transporter in plant development rather than a role in salinity tolerance in plants. Our results suggest that absence of this transporter would alter the thylakoid proton motriz force (pmf) by preventing the dissipation of the membrane potential (Δψ), and possibly, alterations in non-photochemical quenching (NPQ).

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Diversity of barley genetic resources for prompt seed germination after flooding events

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Keywords: dormancy, hypoxia, seed germination

Climate change has dramatically increased high precipitation levels during the crops' growing season that may result in soil flooding events. They are major constraints for crop growth and can result in severe yield losses. Barley is one of the most important cereal crop species worldwide and long-lasting flooding events can affect germination. To identify the genetic diversity involved in the capacity of barley seeds to germinate after a flood event, we used genomic analysis on a large barley panel. The genetic associations between barley landraces and wild relatives with rainfall regimes in their original growing areas were explored. We also explored the contribution to this trait of the bacteria microbiota through metataxonomic studies. Using environmental genomic and molecular approaches, we concluded that genes involved in seed morphology and dormancy are crucial for barley germination in the soil after a flooding event in a natural environment. These results shed light on the complexity of the trait and suggest the importance of the integration of different experimental approaches to study the adaptation of plants to a changing environment.

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Impact of climate change on peach fruit development and postharvest quality: Insights from the warmpeach project

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Keywords: climate change, peach, fruit development, postharvest quality oxidative stress, transcriptomics, adaptation strategies

Peach (*Prunus persica* L. Batsch) is a key global fruit crop, with Spain as the top European producer and exporter. While optimal climatic conditions have supported the industry, climate change threatens fruit production, quality, and market competitiveness. Rising temperatures and extreme weather events impact physiological processes such as flowering, fruit set, and ripening, affecting both yield and postharvest quality. The WarmPeach project evaluates the impact of rising spring and summer temperatures on peach development and postharvest physiology. By monitoring changes in fruit growth, biochemical composition, and susceptibility to physiological disorders and fungal infections, the project aims to develop adaptation strategies for climate-resilient peach production. Data from Spain's National Peach Reference Collection and field trials provide insights into environmental influences on fruit quality, supporting sustainable agricultural practices. A

simulation experiment was conducted on 'Big Top,' 'Nectaperf,' and 'Extreme July' peach trees during 2023–2024. Warming chambers increased temperatures by 4ºC ± 1ºC, allowing controlled study of heat effects on fruit development. Forced heating systems and temperature sensors ensured precise monitoring. Increased temperatures advanced the harvest date by 4–6 days across cultivars, though no significant effects on firmness were observed. In 'Big Top,' higher temperatures resulted in an increase in titratable acidity (TA) and a decrease in soluble solids content (SSC), suggesting metabolic alterations. At both preharvest and postharvest stages, higher temperatures led to elevated reactive oxygen species (ROS) and malondialdehyde (MDA) levels, indicating oxidative stress and membrane lipid peroxidation. While fruit growth was maintained, rising MDA levels suggested incomplete stress compensation, potentially affecting sugar accumulation and organic acid degradation. Further analyses will assess additional quality parameters and investigate gene expression changes linked to stress responses. RNA-seq studies will explore transcriptomic shifts in four cultivars (two melting, two non-melting). The project will also examine how increased temperatures influence postharvest disorders, such as chilling injury (CI) and corky spot (CS), and susceptibility to fungal infections, particularly brown rot (*Monilinia* spp.).

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O-63

Plants under stress: Uncovering the role of miRNAs in rice adaptation to ionizing radiation

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Keywords: ionizing radiation, *Oryza sativa*, *miR169f*, RNAseq

Ionizing radiation (IR) can cause a wide range of detrimental effects on organisms, such as DNA damage and oxidative stress due to the generation or reactive oxygen species (ROS). These effects are dose- and time-dependent, as well as species-specific. Because of long-lived radionuclides, environmental nuclear contamination lasts for hundreds of years, rendering local species chronically exposed to IR. Such an impact may act synergistically with other natural stresses, thus directly affecting plants' growth, development and yield. Therefore, it is critical to understand the mechanisms governing plant responses to IR and the impact of radiation exposure on plant development. In this regard, preliminary results indicated that the modulation of gene expression upon IR exposure also involves the activity of microRNAs (miRNAs). These small RNAs are fundamental controllers of stress responses and of development in plants, and are differentially regulated upon IR exposure in rice (*Oryza sativa*). Despite this, the importance of miRNA-dependent responses to genotoxic stresses has not been carefully explored yet, and the role of miRNAs in plants' adaptation to IR is still largely unknown.

Rice is a monocotyledonous model organism for molecular research, and it is one of the most valuable crop species, being also cultivated in radiation affected areas in Japan. In this study, a transgenic rice line overexpressing miR169f (*OE-miR169f*) and its wild-type counterpart were used to assess the involvement of miRNA/target gene modules on adaptive responses to gamma-irradiation. To investigate the early kinetics of miR169f-dependent responses, we conducted a time-course irradiation at 238 mGy/h for up to 24 h, followed by an evaluation of recovery at the same time points. Physiological traits, as well as targeted gene and miRNAs expression were analyzed. Overall, the shoot weight of *OE-miR169f* was lower than that of its wild-type counterpart, and it was negatively affected by irradiation. Several DNA repair genes (e.g., *SGL* and *RAD51A2*) were upregulated in the irradiated samples after 6 h and 24 h of irradiation exposure. Transcriptome analysis performed on total RNA isolated from shoots and roots irradiated for 24 h revealed different response patterns depending on the organ. The projected outcomes of this study will contribute to the improvement of the current limited knowledge on the post-transcriptional mechanisms behind fast plant adaptation to IR, including insights for improving crops' performance on radiologically contaminated fields.

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From sea to soil: *Saccharina latissima* as a natural soil amendment

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Keywords: drought stress, soil amendment, plant water relation

As climate change intensifies drought events and reduces agricultural productivity, there is an urgent need for sustainable strategies to enhance soil resilience and water retention. The use of macroalgae as soil amendments may offer a dual advantage: it provides an eco-friendly solution for biomass disposal while utilising the hydrophilic properties of alginate-rich cell walls to enhance soil water dynamics.

This study explores the potential of *Saccharina latissima*, a brown alga known for its high alginate content, to improve soil water retention and drought resilience in *Capsicum annuum* L. (pepper plants). A preliminary soil experiment using 22 g/kg of *S. latissima* revealed that treated soil dehydrated more slowly than the control, highlighting its ability to retain moisture.

Physiological analyses further confirmed these benefits: *C. annuum* grown in *S. latissima*-treated soil exhibited significantly more negative osmotic potential at full turgor and turgor loss point, higher stomatal density, and increased gas exchange compared to controls. Accordingly, plants grown in *S. latissima*-treated soil demonstrated greater resilience to drought and an improved ability to recover.

By enhancing soil hydraulic properties and plant resilience, *S. latissima* emerges as a promising and sustainable amendment for agriculture in water-limited environments. This approach not only addresses the growing challenge of drought stress but also promotes the valorization of macroalgal biomass, turning a waste management issue into a climate-smart agricultural solution.

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O-65

Old but gold: Exploiting the underutilized oilseed *Camelina sativa* to uncover and promote tolerance to abiotic stress for improving climate resilience in crops

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Keywords: climate-smart agriculture, crop plasticity, stress resilience

Camelina sativa (camelina, gold-of-pleasure) is an old, re-emerging European oilseed crop without intense breeding history. Camelina is characterized as a low-input crop for bioenergy as well as for food and feed that can be grown on poor soils and marginal land, and it shows inherent tolerances towards adverse environmental conditions such as heat and drought. Thus, camelina is a promising source of stress resilience mechanisms and has a great potential as a climate-smart crop needed to cope with climate change-driven challenges in agriculture. To uncover camelina's stress adaptation strategies, the EU-Horizon 2020 project UNTWIST [1] (GA 862524) is using an interdisciplinary systems approach to dissect its multi-layered responses to heat and drought. Screening the performance of 54 genetically diverse lines in field trials as well as under controlled heat and drought conditions combined with genomic and metabolomic analyses allowed the selection of four contrasting focus lines. These lines were further characterized in additional field trials under different cropping systems to analyse their agronomic performance, and in complementary large-scale experiments under controlled heat and drought conditions for in-depth mechanistic analyses. Results of

the four focus lines from a controlled drought stress experiment in an automated high-throughput phenotyping setup will be presented in detail. This approach allowed to discriminate differential behaviour of the focus lines under well-watered and drought conditions at multiple morphological and physiological levels through RGB, hyperspectral reflectance, chlorophyll fluorescence and thermal imaging techniques. These results will be integrated with (epi)genomic, physiological, metabolic, proteomic, and transcriptomic data to feed into mechanistic and predictive models and to derive markers for crop improvement. Moreover, implications for the understanding of genetic interplay and plasticity in plant adaptation, which can be exploited for increasing crop yield stability in adverse and changing environmental conditions, will be discussed.[2]

[1] https://www.untwist.eu

[2] Großkinsky et al. (2023) The potential of integrative phenomics to harness underutilized crops for improving stress resilience. Front Plant Sci 14:1216337.

O-66

Brassinosteroid seed-priming enhances the photosynthetic efficiency of *Sorghum bicolor* salt-sensitive hybrid

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Keywords: salt-stress, brassinosteoids, *Sorghum bicolor*

The salinization phenomenon is increasing and spreading worldwide, negatively affecting ecosystems and agriculture. Thus, understanding the mechanisms that allow salt-tolerant crops to survive and to be productive in this changing environment is crucial for ensuring food availability. Besides understanding these mechanisms, it is essential to find strategies that help crops to cope with harsh abiotic stresses, such as salt stress, without harming ecosystems. *Sorghum bicolor* is a salt-tolerant crop, although differences among genotypes were found (2). It represents a stable food in many arid and semi-arid regions of the world, thus it is a good candidate for studying salt-tolerance mechanisms. Brassinosteroids are a class of steroidal hormones well known for their role in plant development and tolerance to different stresses, including salt-stress. Their exogenous application, also as seed-priming, could improve stress tolerance with minimum impact on the environment (1). This study aimed to verify if the seed-priming treatment, with the brassinosteroid 24-epibrassinolide (eBL) at 1 µM, could enhance salt resistance in a salt-tolerant and a salt-sensitive *S. bicolor* hybrids, namely Bianca and Tonkawa. The results obtained from 40-days-old in-pot cultured plants highlighted the efficacy of the eBL seed-priming treatment in enhancing the photosynthetic efficiency of the sensitive genotype Tonkawa. In fact, gas exchange analyses showed a significant increase in net photosynthetic rates and a decrease in intercellular CO_2 content, leading to higher carboxylation efficiency. Shoot morphological analyses showed an amelioration in Tonkawa's parameters under saline soil conditions, when treated with eBL priming. Histological analyses highlighted more differences among treatments in Tonkawa, with respect to Bianca, especially between plants treated only with salt and those pre-treated with the hormone. Altogether, the results show that seed pre-treatment with a specific eBL concentration can increase salt tolerance in the salt-sensitive genotype.

[1] Anwar et al., (2018). Biol. Res. 51, 46. https://doi.org/10.1186/s40659-018-0195-2

[2] Peduzzi et al., (2024). Env. Exp. Botany 226. https://doi.org/10.1016/j.envexpbot.2024.105876

O-67

Guard cell metabolism – A key for regulating drought resilience?

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Keywords: drought stress, stomata, metabolites

In view of increasing drought frequencies due to climate change, enhancing crop resilience to water scarcity has become vital for sustainable agriculture. This study examines drought adaptation strategies in maize and grapevine guard cells. We compared the maize guard cell metabolome with grapevine guard cells under comparable controlled drought conditions. Physiological and metabolomic analyses were conducted using mesophyll and guard cells under single and repeated drought stress conditions. An untargeted metabolic

profiling was performed by using GC-MS. Grapevine guard cells exhibited stable amino acid and sugar profiles, while maize guard cells showed dynamic sugar accumulation. These responses illustrate two contrasting strategies, namely a muted acclimation of grapevine and a vigorous acclimation of maize in mesophyll and guard cells. In both crops, metabolite concentrations were less impacted by drought stress in guard cells compared to mesophyll cells, suggesting the emphasis of plants to maintain stable guard cell metabolomes for functional integrity. Furthermore, supplemental sulfate promoted enhanced metabolic adjustments in mesophyll cells under drought conditions, while the effect of supplemental sulfate in guard cells was markedly diminished. Despite their contrasting drought strategies, grapevine and maize exhibited similar responses to supplemental sulfate. This suggests that sulfate fertilization could be a viable strategy to improve the resilience of crops to drought stress. In conclusion, this study identified the contrasting drought adaptation strategies of grapevine and maize, highlighting the critical role of guard cell metabolic stability in maintaining functional integrity under drought conditions.

■

O-68

Effect of long-term stress memory on the regulation of grapevine responses to repeated drought events

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Keywords: eco-physiology, drought priming, transcriptome reprogramming, epigenetic signature

Plants have evolved different strategies to cope with environmental stresses and, although still debated, it was observed that they can remember past stress occurrence. Anatomical and physiological adjustments were observed in different grapevine cultivars after repeated drought exposure, however epigenetic, transcriptional and biochemical changes associated with drought-primed ecological memory still need to be explored.

In this work, we test whether exposure to recurring mild drought events could prime vines to endure severe drought stress. We therefore investigated whether the expected improved stress tolerance of *Vitis vinifera* cv Nebbiolo plants subjected over years to moderate and long-lasting water stress events (WS-primed) depended on molecular memory phenomena or on resetting of stress-induced signals. A combined multidisciplinary approach, involving eco-physiological, anatomical, biochemical and molecular analyses, was adopted. Physiological results showed that WS-primed vines had reduced gas exchange in well-watered conditions, but at the end of WS imposition were able to maintain higher transpiration and assimilation rates with respect to unprimed plants. Though at the anatomical level, no significant differences were observed by analyzing current-year sprouts, WS-primed plants accumulated lower amounts of root abscisic acid and had higher content of resveratrol and viniferin, suggesting an enhanced antioxidant capacity. Whole transcriptome changes inspected in roots and leaves also suggested the establishment of specific molecular signals following repeated exposure to water stress. Further analyses are ongoing to identify specific associations between key epigenetic marks and transcriptome and physiological changes.

In a future perspective, the gained information will deliver a predictive framework to estimate the impact of moderately dry periods on vine performance, considering memory-associated protective effects against drought.

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■

O-69

Regulation of root meristem size in rice by cytokinins and ROS under salt stress

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Keywords: Root Apical Meristem (RAM), salinity stress, rice root plasticity

The root is a fundamental organ for plant nutrition, anchoring, and perception of soil-borne stresses. Root structure, influenced by genetic and environmental factors, is dynamic and modulated during plant growth in response to environmental conditions (plasticity). This plasticity is linked to the activity of the root apical meristem (RAM), regulated by hormones and reactive oxygen species (ROS) in *Arabidopsis*. Salinity inhibits root growth, reduces root hair density, and alters root system architecture, leading to biomass loss and decreased productivity. Given that rice is predominantly cultivated in coastal areas prone to salinization due to drought or flooding, understanding the molecular mechanisms governing RAM development and maintenance under salt stress is crucial for combating productivity loss.

Our study leverages the conservation between *Arabidopsis* and rice RAM to translate knowledge across species. We identified similar mechanisms of meristem size determination. Imaging of the rice seminal root at the cellular level revealed that transition zone (TZ) positioning, and thus meristem size, is influenced by cytokinins, ROS, and high salinity. We observed parallels in the distribution of H_2O_2 and O_2^-. Additionally, we identified homologous genes in rice, including PINs, RRs, and GH3s, which exhibited differential expression in response to cytokinins and H_2O_2. Comparing salt-sensitive and salt-tolerant rice varieties, we found that tolerant plants maintained RAM size under stress by modulating cytokinin and auxin responses.

In conclusion, our findings demonstrate that similar mechanisms regulate RAM size in rice and *Arabidopsis*, providing a foundation for understanding rice root plasticity under high salinity conditions–a major threat to global rice productivity.

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O-70

miRNA-based regulatory networks: A key to enhancing salt tolerance in rice

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Keywords: salt tolerance, miRNA regulation, rice

Soil salinization, driven by human activities and climate change, poses a major threat to agricultural productivity, particularly in rice (*Oryza sativa*), the world's most important staple crop. Identifying molecular regulators and biomarkers of salt tolerance is key to developing more resilient and productive varieties. Among these, microRNAs (miRNAs) have emerged as critical modulators of plant stress responses.

This study investigates miRNA-dependent regulatory mechanisms underlying salt tolerance in over ten Italian rice varieties (*Oryza sativa* subsp. japonica) of high agronomic value. We screened these rice varieties for salt tolerance during seed germination and early seedling establishment, classifying them based on key phenotypic and biochemical traits. Using the two most tolerant and two most sensitive varieties, we conducted sRNA-sequencing to identify miRNAs early responsive to salt stress in leaf. By integrating these findings with *in-silico* analyses and gene expression profiling, we uncovered two novel regulatory modules involved in the early response to salt stress.

The first module, miR167h-WAK4 (Wall-associated kinase 4), responds strongly to salt exposure in both tolerant and sensitive varieties. miR167h is consistently downregulated, resulting in increased WAK4 levels following short-term salt stress. Given WAK proteins' role as cell wall integrity sensors interacting with pectins, this module may play a critical role in linking cell wall dynamics with intracellular signaling, thus influencing leaf development under salinity. The second module, miR530-RMT1 (Microtubule-Associated RING Finger Protein), is exclusively regulated in the tolerant variety, suggesting its role in enhancing salt tolerance. RMT1, the predicted miR530 target, was differentially expressed between tolerant and sensitive varieties, indicating its potential function as a salt-responsive protein. To further characterize the miR530-RMT1 module under salinity, we

also examined PIL15, a transcription factor regulating miR530 expression.

This study provides new insights into the molecular basis of salt tolerance, offering potential targets for breeding salt-resilient rice varieties.

This work was supported by the National Research Program and for Projects of National Interest (NRP) PRINN grant (MIR-SeeD: MIcroRNA signature modulating Salinity and Drought tolerance in rice seeds).

O-71

Combined and isolated effects of water deficit and salinity on *S. Habrochaites* and *S. Galapagense*: A physiological approach

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Keywords: combined abiotic stress; photosynthesis; salinity; wild tomato species

Soil salinization and water deficit, worsened by climate change and unsustainable farming, are two major factors reducing crop yield. While greenhouse farming can mitigate environmental stress, vulnerable outdoor cultivation remains dominant in the world's most producing regions. Our study, simulating realistic field conditions, aimed to compare the effects of single and combined stress from salinity and water deficit on wild tomato species (WTS) *Solanum galapagense* (SG) and *Solanum habrochaites* (SH) vs. the domestic tomato *Solanum lycopersicum* (SL). WTS showed greater stress resilience, despite their slower early growth, than SL. The domestic cultivar showed significant reductions in shoot and root growth under stress while both WTS maintained root growth. Combined stress reduced WTS's shoot growth, however, SG leaves remained unaffected by the low irrigation treatment alone, and SH sustained shoot growth under single salt stress. Chlorophyll and carotenoid levels steadily declined in SL, whereas pigment content increased in SH plants under stress. Results on photosynthetic induction and stomatal conductance indicated that SG was the only genotype significantly affected by combined stress in terms of photosynthetic efficiency, possibly due to pigment loss and stomatal closure, despite not exhibiting the greatest growth inhibition. Unlike SL, WTS increased root amino acid and sugar content under combined stress and had higher baseline amino acid levels. Nitrate content was constitutively higher in SH leaves and SG roots but generally decreased under salt and combined stress. SG exhibited the highest leaf glutamine synthetase activity, although this declined under stress. Ammonium levels remained unaffected by stress in SG, increased in SH roots under combined stress, but decreased in the cultivar's roots. Overall, growth inhibition in SL likely resulted from osmotic stress, which may have affected water and nutrient uptake, cell expansion, and hormonal balance, rather than directly impairing the efficiency or structure of the photosynthetic apparatus. Additionally, salt exposure may have triggered premature senescence and oxidative stress. The superior root growth, osmotic regulation, tighter stomatal control, and more efficient nitrogen metabolism of WTS likely contributed to their higher stress tolerance.

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O-72

Biostimulants derived from plant cell cultures used as an alternative for ameliorating abiotic stress tolerance in brassica seeds

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Keywords: higher plant-derived biostimulant, Brassica seeds, orange carrot cell culture

Facing the increasing antropogenic pressure and adverse edaphoclimatic conditions derived from climate change is the main challenge for modern agriculture. This makes necessary to elaborate new alternatives to increase crop yield without causing significant harm to the environment. Biostimulants, among which higher plant-derived biostimulants (hPDBs) stand out, have been proved to alleviate the effects of abiotic stress on plants growing under unfavorable conditions by modulating plant physiological processes[1]. However, the composition of hPDBs is often heterogeneous, as its composition

depends on harvest season and the variety of the source used for its production. In this work, we evaluate the potential of an orange carrot cell culture-derived biostimulant (OCB), a novel type of hPDB based on plant cell cultures, as they constitute stable production systems of bioactive compounds. OCB was applied as priming agent in Brassica seeds subjected to salinity stress, with the goal of producing more competitive and tolerant seedlings to the effect of climate change and improve their vigour and quality. Finally, we describe its positive effect on germination parameters and oxidative stress markers, as well as its impact on the hormonal profile of the seedlings developed. Thus, OCB constitutes a novel biostimulant with potential to alleviate salinity stress in Brassica seeds.

[1] Martínez-Lorente, S.E. et al., Antioxidants, 2024; https://doi.org/10.3390/antiox13030318.

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O-73

PEG-triggered osmotic stress generates large-scale transcriptional and epigenomic changes in rapeseed (*Brassica napus* L.)

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Keywords: Rapeseed, ChIP-seq, RNA-seq, DNA methylation, drought response, P5CS genes, proline accumulation

Physiological and molecular background of plant responses to drought has already been extensively studied. Epigenetic regulation of gene expression in crop plants in response to dehydration is however less known. We deciphered genome-wide changes in transcriptome and histones modifications in response to dehydration in rapeseed (*Brassica napus L.*). High-throughput transcript profiling (RNA-seq) and chromatin immunoprecipitation followed by sequencing (ChIP-seq) of PEG-treated rapeseed plants revealed genome-scale changes in transcription and histone methylation patterns. Changes in histone H3 lysine 4 trimethylation (H3K4me3) and histone H3 tri-methylated lysine 27 (H3K27me3) sites were characterized. Gene sets with altered transcript profiles as well as histone methylation marks in response to osmotic stress were identified. PEG-triggered osmotic stress lead to significant proline accumulation. Several proline biosynthesis regulatory genes coding for Delta 1-Pyrroline-5-Carboxylate Synthetases (P5CS) were induced by PEG treatment and displayed changes in H3K4me3 and/or H3K36me3 enrichment. Targeted bisulphite sequencing further identified stress-dependent gene body DNA methylation in one of the BnP5CSA gene copies that correlates with its stress-induced activation. Integration of physiological, transcriptional and epigenomic data could contribute to a better understanding of the drought response control in crop plants such as rapeseed.

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Prasad, M, Shetty P, Pal AK, Rigó G, Kant K, Zsigmond L, Nagy I, Shivaprasad PV, Szabados L (2025) Transcriptional and epigenetic response of rapeseed (Brassica napus L.) to PEG-triggered osmotic stress. J Exp Bot (JEXBOT-2024-313959v2, in press).

O-74

A novel regulatory step of the miRNA pathway

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Keywords: miRNA, loading efficiency, precursor structure

RNA interference mediated via the action of micro RNAs (miRNAs) plays pivotal role in developmental and stress response pathways. In the nucleus, plant miRNAs are generated by subsequent enzymatic cuts of the MIRNA precursors, having specific hairpin-like secondary structures, to liberate the miRNA/miRNA* duplex. The mature miRNA strands are then loaded mostly into the

ARGONAUTE 1 containing RNA induced silencing complex (RISC) and trigger the down-regulation of the expression of the target mRNAs, while the miRNA* strands are eliminated. We identified a novel regulatory step of the pathway, where the extent of the target mRNA down-regulation depends not solely on the miRNA concentration, but on the AGO-loading efficiency of the miRNA in the investigated plant material. Different miRNAs have various loading properties, which is independent of their abundancy. We identified the competitive manner of miRNA loading where the AGO level is the limiting factor. Using transient and transgenic expression studies of artificial and chimeric miRNA precursor constructs we revealed that MIRNA precursor structural elements like the miRNA/miRNA* duplex and the surrounding region, can have influence on the AGO-loading efficiency of the produced miRNAs. This effect was the most pronounced when the orientation of the duplex was also considered. This implies the cooperative action of the different structural elements. The discovery of signals on remote structures of the miRNA precursor suggest that miRNA biogenesis and AGO-loading can be spatially more connected in nucleus and/or signalization events mediated by these non-duplex structural features during miRNA biogenesis can determine the fate of the miRNA/miRNA* duplexes in separated AGO-loading processes. According to our hypothesis the AGO-unloaded miRNA pool could serve as a basis for rapid counteraction against environmental stresses.

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O-75

Physiology shaped by environmental cues: Deciphering annual growth-dormancy cycle in Norway spruce (*Picea abies*)

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Keywords: Norway spruce, dormancy, development, epigenetics, cold acclimation

Norway spruce (*Picea abies*), like many other long lived conifers, undergo annual physiological phase shifts by entering dormancy in the late summer-early fall and transit from the latent phase to the growth phase in late spring. From this time onwards, the active growth is continued during the summer. Although this phenomenon is an extensively documented physiological trait, the molecular mechanisms behind creating and maintaining such 'switch' are not clear. Moreover, the connecting factors bridging the environmental signals and establishing timely phase shift, creating such minute regulation, are not well understood. In this study, we aimed to elucidate the transcriptome wide changes related to growth-dormancy phase shifts happening throughout the year to find correlation with the seasonal changes. Since, the epigenetics is a significant contributer in the control of important physiological processes such as bud burst, bud set, de-hardening, and cold acclimation, we aim to connect the changes in the growth-dormancy related gene expression with other regulatory factors such as DNA methylation status of such genes and small RNA-mediated regulation. Together, the results will reveal significant novel molecular interactions deciphering the annual growth-dormancy phase shifts in Norway spruce, and may provide valuable information on how plant's adaptation could be affected by today's changing climate.

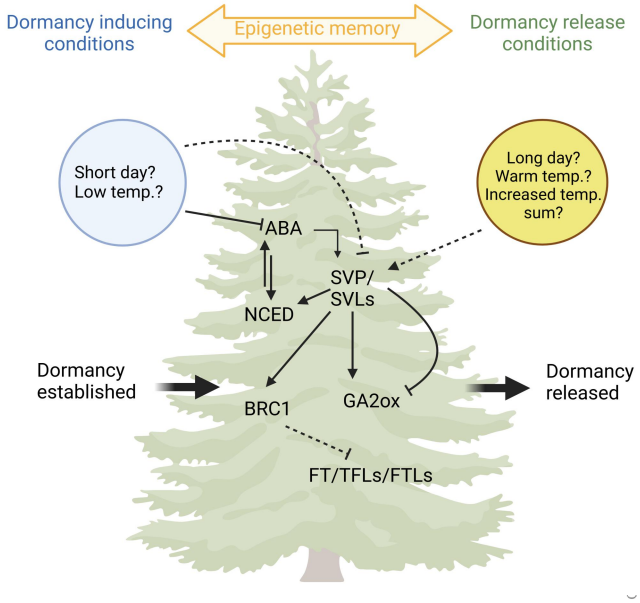

Figure 1: *A putative pathway causing dormancy induction and release in Norway spruce.*

O-76

Pros and cons of targeted and high-throughput *BPM2* mRNA isoforms detection in *Arabidopsis thaliana*

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Keywords: alternative splicing, MATH-BTB, Sanger sequencing, whole-transcriptome sequencing

MATH-BTB proteins are involved in the proteasomal degradation pathway as specific substrate adaptors of CUL-3-based E3 ubiquitin ligases. By modulating protein turnover, they regulate plant development and abiotic stress response. The *MATH-BTB* gene family is significantly expanded in grasses, with genomes of different grass species containing dozens of different *MATH-BTB* genes. In contrast, the *Arabidopsis thaliana* genome only contains six *MATH-BTB* genes (*BPM1-6*). However, all *BPM* genes contain introns facilitating posttranscriptional mRNA processing, while most of the grass *MATH-BTB* genes are intronless. The aim of this work was to identify and characterize novel splice variants of the *A. thaliana* gene *BPM2*. A bioinformatic analysis of the latest *A. thaliana* transcriptome, AtRTD3, revealed that all *BPM* genes undergo alternative splicing and encode a total of 56 transcript isoforms that may code for 38 different putative proteins. Of those, the *BPM2* gene is transcribed in 16 transcript isoforms and probably encodes 9 different putative proteins – substantially more than the five *BPM2* transcript isoforms currently indexed in TAIR. To experimentally validate the TAIR indexed and novel *BPM2* isoforms, universal and variant-specific primers were designed based on a multiple sequence alignment of all 16 *BPM2* isoforms. RT-PCR confirmed the presence of several novel *BPM2* transcripts from AtRTD3 in different *A. thaliana* tissues. Whole-CDS fragments amplified with universal primers were cloned into plasmids and sequenced with Sanger sequencing. The vast majority of *BPM2* amplicons corresponded to *BPM2.12*, and none of them represented the novel isoforms from AtRTD3. However, several splice variants that aren't included in any existing database were detected in different tissues by whole-CDS Sanger sequencing. This result indicates differences in the ability to detect low-abundance transcripts using targeted and general sequencing approaches.

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O-77

GOLEM: A computational tool for exploring Gene regulatOry eLEMents across the plant tree of life

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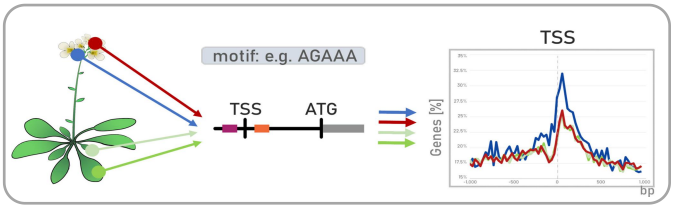
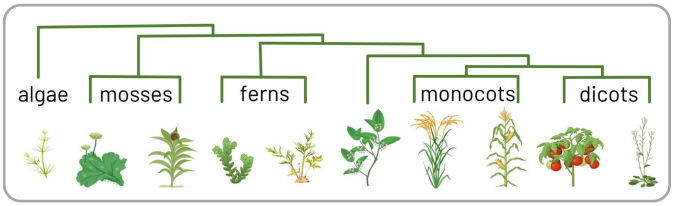
Keywords: promoters, CREs, transcription, male gametophyte

GOLEM (Gene regulatOry eLEMents, golem.ncbr.muni.cz) is a user-friendly tool which allows browsing plant genomes within the plant Tree of Life, encompassing streptophyte algae, mosses, ferns, basal angiosperms, monocots, and dicots. The GOLEM enables the analysis of various plant tissues, including sporophytic structures (e.g., leaves) and male gametophytic developmental stages (e.g., antheridia, pollen stages, and sperm cells). The GOLEM tool enables to investigate the precise localization and distribution of any CREs of interest in gene promoters, in proximity to the TSS and ATG. The set

of investigated genes can be specified by the level of gene expression in specific tissues based on transcriptomic data. Furthermore, tracking of the genome-wide distribution across exemplified genomes, regardless of the transcription level, may aid to track the evolution of regulatory motifs across the plant Tree of Life.

We will demonstrate the utility of GOLEM by analyzing motifs associated with male gametophyte development (e.g., LAT52, MEF2, and DOF_core), hormone-responsive elements (e.g., GCC-box, ARR10_core), and conserved regulatory motifs (e.g., TATA-box, ABRE, TC-element, I-box, and DRE/CRT element), among others.





This work was supported for by the Czech Science Foundation [21-15841S] and by the Ministry of Education, Youth and Sports of the Czech Republic under the project INTER-COST LUC24056.

Nevosad et al., TPJ, 121, 5, 2025 doi.org/10.1111/tpj.70037

O-78

Genomic variation in the Andean lupin (*Lupinus mutabilis*): Genome annotations, structural variations, and utility for breeding

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Keywords: Andean lupin, genomic variation, breeding

Andean lupin (*Lupinus mutabilis*) is a high-protein leguminous crop native to the Andean region, traditionally cultivated in Ecuador, Peru, and Bolivia. Its seeds contain up to 44% protein (dry matter) and 18% oil, making it a promising plant-based protein source. In addition to its exceptional nutritional profile, *L. mutabilis* exhibits strong agronomic resilience, thriving in high-altitude environments and low-input agricultural systems due to its nitrogen-fixing capacity. However, despite its potential as a climate-resilient and nutrient-rich crop, Andean lupin remains largely underutilized and under-researched. Several barriers hinder its broader adoption, including the presence of toxic quinolizidine alkaloids. Genomic resources are essential for advancing *L. mutabilis* breeding efforts, and the recent release of the *L. mutabilis* reference genome has provided valuable insights into key agronomic traits, including alkaloid biosynthesis and disease resistance[1]. However, the genetic diversity within *L. mutabilis* remains uncharacterized, particularly regarding structural variants (SVs), which may be influencing phenotypic diversity and adaptation. In this study, we employed ONT sequencing to generate and assemble draft genomes for four *L. mutabilis* accessions, including the commercial variety INIAP-450 Andino and three Ecuadorian accessions with distinct agronomical traits. Given that low alkaloid content in Andean lupin seeds is a primary breeding goal, we further analyzed genes associated with alkaloid biosynthesis. A BLAST search across the four *L. mutabilis* genomes identified candidate genes previously linked to alkaloid production (RAP2-7, HMT/MLT, LaCAO) in other cultivated lupins. Comparative analyses revealed that bitter *L. mutabilis* accessions retained specific amino acid residues at key positions associated with high alkaloid production in *L. angustifolius* and *L. albus*. Additionally, we investigated two specific SVs in genes of the sweet *L. mutabilis* variety Inti, proposed as candidates for explaining its extremely low alkaloid concentration. The first gene, ANN14873, is a member of the RAP2-7 family, syntenically conserved across all lupins, and carries a premature truncation leading to the loss of two APETALA2 protein domains, which are conserved in *L. albus* and *L. angustifolius*. The second gene, ANN30223, encodes an HMT/HML protein containing a large insertion (334 amino acids) within the transferase catalytic domain, substantially altering the protein structure and resulting in a loss of function. Our analysis showed that none of our four bitter accessions carried the premature truncation in ANN14873, while the transferase catalytic domain of ANN30223 remained intact in all analysed bitter accessions. Our findings not only expand the genomic resources available for *L. mutabilis* but also offer insights into the genetics of alkaloid biosynthesis, a crucial trait for Andean lupin breeding programs. By integrating long-read sequencing and comparative genomics, this study advances our understanding of the genetics of key agronomic traits and provides valuable tools for breeding programs. Ultimately, these efforts could contribute to the breeding of Andean lupin, benefiting both Andean farming

communities and global markets seeking alternative protein-rich crops.

[1] Pancaldi, F., Gulisano, A., Severing, E. I., van Kaauwen, M., Finkers, R., Kodde, L., & Trindade, L. M. (2024). The genome of Lupinus mutabilis: Evolution and genetics of an emerging bio-based crop. The Plant Journal, 120(3), 881-900.

O-79

On the building of PlantaeViz, an integrated omics platform for non-model and crop plant species

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Keywords: data visualization, web tools, genomics, transcriptomics

The development of omics platforms for non-model plant species lags behind those available for model species, limiting researchers' access to integrated genomic and transcriptomic data. While public repositories host an increasing number of datasets spanning diverse species, a centralized platform is needed to facilitate data exploration and analysis. To address this, we he have developed PlantaeViz, a suite of species-specific platforms providing advanced visualization and analysis tools, including gene cards, gene expression atlases, BLAST searches, multiple sequence alignments and gene co-expression and regulatory networks. PlantaeViz currently supports major crop plants (e.g., grape, tomato, cannabis) and model species such as Arabidopsis, offering user-friendly navigation and access to extensive RNA-Seq datasets exceeding 10,000 processed runs for some species. A custom ontology framework classifies public transcriptomic data by tissues, developmental stages, and other biologically relevant categories, enabling intuitive and interactive exploration. The GeneCards module integrate curated gene catalogues with functional annotations and expression profiles, while JBrowse2 genome browsers and BLAST servers facilitate comparative analyses, including syntenic data, to enhance cross-species interoperability. Additionally, newly generated condition-dependent and independent gene co-expression (GCNs) and regulatory networks (GRNs) enable users to identify top co-expressed and regulated genes and perform gene set enrichment analyses (e.g., MapMan, GO). A 3D interactive module, built with networkD3, allows for dynamic network visualization of custom gene sets, aiding in the identification of key regulatory genes and pathways. PlantaeViz provides an integrated, modular and scalable platform designed to support a broad range of plant species, bridging the gap between model and non-model plants.

O-80

Genome-wide association study reveals novel QTLs associated with lignan content in sesame accessions from the RDA-genebank

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Keywords: sesame, GWAS, germplasm, lignan

Sesame (*Sesamum indicum* L.) is primarily cultivated for its seeds, which comprise 44–58% oil, 18–25% protein, and approximately 13.5% carbohydrates. In addition to its nutritional attributes, the crop is highly valued for its rich lignan content. Lignans, phenolic compounds synthesized from two phenylpropanoid molecules, are known to confer beneficial effects on human health by reducing blood glucose and cholesterol levels. The RDA-Genebank in the Republic of Korea conserves nearly 8,000 sesame accessions from around the world. Based on origin data, 300 sesame accessions representing 31 countries were selected and cultivated over two years (2021 and 2022) at the RDA-Genebank field. Harvested seeds were analyzed for lignan content using high-performance liquid chromatography (HPLC), revealing a two-year average total lignan content ranging from 1.17 to 11.54 mg/g. Concurrently, whole-genome resequencing identified 2,032,196 high-quality single nucleotide polymorphisms (SNPs) distributed across all

chromosomes and scaffolds. A genome-wide association study (GWAS) identified four quantitative trait loci (QTLs) associated with lignan content, located on chromosomes 4, 6, 11, and 13, which harbor seven promising candidate genes. Moreover, an additional candidate gene on chromosome 6 spans three linkage disequilibrium (LD) blocks. The elucidation of these novel QTLs and candidate genes provides valuable targets for breeding programs aimed at enhancing the functional properties of sesame. Further functional validation of these genomic regions is expected to accelerate the development of sesame varieties with improved nutritional and health-promoting qualities.

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■

O-81

Utilisation of high-throughput phenoytping platform in Martonvásár

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Keywords: cereals, phenotyping, multispectral sensors

In 2023, a new phenotyping system was installed in the Agricultural Institute, Centre for Agricultural Research (ATK), HUN-REN, in Martonvásár. This new phenotyping platform consists of cc. 200 m^2 growth chambers and a measuring chamber. In growth chambers, the environmental conditions can change within a wide range including temperature (10-35 °C), light intensity (0-1500 µmol m-2 s-1) and spectral composition due to the LED light sources and CO_2 level till up to 1500 ppm. The growth chambers are equipped with an automatic watering system enabling to water the plants either with water or with mineral nutrition. By controlling the water withdrawal, the effect of drought stress can be examined separately or together with other environmental stresses (e.g. heat).

In the measuring chamber, 5 boxes are placed enabling separate detection of several morphological and physiological parameters, including chlorophyll *a* fluorescence and infra-red signals, making RGB images and 3D structures via laser scanning, as well as the reflectance of light through hyperspectral analysis. The chlorophyll fluorescence sensor helps in the investigation of photosynthetic electron transport processes. In addition, the measuring box allows the detection of different fluorochrome dyes such as DAPI, SYBR Green, YFP, GFP. With the use of infrared spectroscopy, the absolute and relative temperature of plants can be determined. The characteristic morphological parameters of plants (plant height, surface area, circumference and colour) can be detected using RGB sensors. The 3D laser scanning detector can be used to create a 3D structural image of plants. Hyperspectral cameras measure the light rays reflected from the plant surface in the 380-1700 nm spectral range, which can be used to characterize numerous metabolic processes.

The high-throughput phenotyping system is mainly used for the cultivation of cereals including wheat, barley or maize to study the effect of adverse environmental conditions on the growth and development and stress response of plants. In addition, the system enables a quick selection of genotypes having useful agronomic traits from wide genetic plant material. All this can contribute to increasing the efficiency of breeding. In the presentation, the operation of a high-throughput phenotyping system will be presented through examples performed in our institute. The phenotyping platform won the EXCELLENT RESEARCH INFRASTUCTURE NETWORK in HUNGARY (3-133-e). The phenotyping platform is available for scientists and breeders within and outside the Institute. Further information is found at our webpage: https://atk.hun-ren.hu/en/phenotyping-system/ or please be in contact with the head of the platform: darko.eva@hun-ren.atk.hu

■

O-82

From pixels to phenotypes: Semi-automated classification of autumn reddening and bud set using hyperspectral imaging in scots pine

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Keywords: phenotyping, hyperspectral imaging, provenances

This study investigated key phenological traits, specifically autumn reddening and bud set, in eight European Scots pine (*Pinus sylvestris* L.) provenances. Autumn leaf reddening (anthocyanin accumulation) and the timing of bud set are responses to environmental stressors such as reduced daylight and cooler temperatures. These traits exhibit substantial genetic variation, influencing species' adaptation to environmental conditions. Our experiment comprised a total of 4366 seedlings in 57 trays. We assigned the provenances to 3 groups corresponding to European regions of similar climatic conditions. All the seedlings were grown in a nursery common garden experiment located in Finland. So far, phenology in forest trees has been assessed via scoring, which can be very subjective and error-prone. Thus, we propose a supervised classification method using hyperspectral images from the VNIR SNAPSCAN camera (imec, Leuven, Belgium). We created a pipeline for the supervised classification using the freeware Spectronon software (Resonon Inc., USA) and Fiji (NIH, USA). The supervised classification method conserves computational power by processing each image (one tray) separately, avoiding the computational burden of appending hyperspectral images together. The supervised classification method establishes a common threshold for classifying individual images by utilizing pixels from all provenances for each class (background, green needles, red needles, dry needles, and buds). We tested the classification results and spectra for provenance group effects using partial least squares discriminant analysis (PLS-DA). The classification results predicted provenance subgroups with 0.95 accuracy (Kappa = 0.92). For comparison, PLS-DA using raw spectra predicted provenance subgroups with 0.82 accuracy (Kappa = 0.73) and first-derivative spectra with 0.93 accuracy (Kappa = 0.89). Although the first-derivative spectra predict the provenance subgroup with comparable accuracy to our classification results, the classification output offers additional insight through graphical visualization of the differences. Moreover, we created a Fiji (NIH, USA) macro counting the terminal buds based on the classification images with high accuracy (F-score = 93%). The proposed high-throughput method allows for time-efficient phenotyping of phenological traits. Due to being semi-automated, it is also less prone to observer bias. The new monitoring approach adaptable to any species reveals how population origin shapes tree adaptation and resilience.

■

O-83

Unraveling the mechanism of action of a biostimulant: A successful integration of ecophysiologycal investigation and high-throughput phenotyping

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Keywords: biostimulant, abiotic stress, high-throughput phenotyping, photosynthesis

Biostimulant (bs) products are a promising technological solution in agriculture, providing a key strategy to sustain crop quality and yield under climate challenges. To maximize product benefits and expand their application scope, beyond the progress made in formulation and characterization, it is essential to develop a structured strategy to study their mechanisms of action, ensuring robustness to account for both bs diversity and crop-specific responses.

In this study, we used combination of ecophysiological analyses and High-Throughput Phenotyping to unveil the performance and mode of action of YaraAmplix OPTIVI (Yara International S.A.), a commercially registered bs under Regulation (EU) 2019/1009, to mitigate the negative effects of water stress effects in grapevine.

Our solid approach indicated that the bs induces structural and functional modifications in leaf and stem tissues, promoting a water-saving strategy under well-watered conditions, while enhancing transpiration and photosynthetic efficiency during water stress. This dual response sustained carbon assimilation and accelerated recovery upon rehydration. The effect was driven by a drastic reduction in water and osmotic potential, triggering an anisohydric response under moderate stress while preserving stomatal control and protecting the photosynthetic apparatus under severe stress. Moreover, starch quantification in shoots revealed that enhanced photosynthetic efficiency led to greater reserve accumulation, improving stress resilience. High-temporal-resolution weighing lysimeters detected transpirative changes just days after the first application, while multispectral 3D imaging analysis identified leaf angle and Green Leaf Index as the most responsive

parameters to water stress, effectively capturing early responses and indicating improved stress tolerance.

Future field research should further assess the ability of bs to enhance plant resilience and performance in applied agricultural contexts while also investigating its potential effects on fruit quality traits and ripening kinetics.

This work was funded by the project 'POC Plant Flow Solutions,' NODES Spoke 2 – Green Technologies and Sustainable Industries (CUP D17G22000150001), and by Yara International S.A., which provided the bs and supported the research trial.

■

O-84

Sunlit insights: Transferring hyperspectral imaging from lab to field

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Hyperspectral imaging has long been a key tool for non-destructive plant phenotyping. Photon System Instruments (PSI) integrates hyperspectral (HS) cameras into phenotyping pipelines, using them as essential data sources to assess plant health and developmental stages in controlled environments. However, research shows that plant responses to environmental stress can vary significantly between laboratory and field conditions, even in genetically identical plant lines, affecting the accuracy of yield and nutritional predictions.

To address this challenge, PSI developed an advanced HS image processing pipeline, tested on a comprehensive dataset of Calendula flowers. This system together with a series of HS image preprocessing steps features a deep learning classifier based on a 1D convolutional neural network, which effectively captures key spectral signatures of each plant pixel despite variations in lighting–an issue that has long hindered the application of field HS imaging for phenotyping. The model achieves high classification accuracy, successfully distinguishing between 10 Calendula lines in field conditions.

This study highlights the advantages of high-speed, high-precision HS imaging, enabling rapid data collection while compensating for environmental variability and noise. Compared to traditional methods like manual sampling, spectrophotometry, or RGB imaging, this approach offers greater reliability, accuracy, and efficiency in field-based plant phenotyping. As a result, this pipeline significantly improves the transition of high-throughput, precise HS phenotyping from laboratory research to real-world field applications.

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O-85

Promising candidates for new pamps - How plants handle bacterial cyclic dinucleotides

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Keywords: cyclic dinucleotides, plant signaling, plant immunity

Cyclic dinucleotides (CDNs) comprise a group of uncommon nucleotides found in bacteria and metazoans that are signaling molecules engaged in various physiological processes. There are three known prokaryotic CDNs, namely c-di-AMP, c-di-GMP and 3'3'-cGAMP, which are readily synthesized and hydrolyzed by specific bacterial cyclases and phosphodiesterases. Animal cells produce their own CDNs (2′3′-cGAMP and 3′2′-cGAMP), but they are also capable of responding to bacterial ones, which induce an innate immunity response upon reception. To date, no reports have indicated the presence or role of cyclic dinucleotides in plants.

Herein, we report a novel discovery that plants (*Vitis vinifera*) carry enzymes that hydrolyze bacterial c-di-AMP, leading to the formation of four possible products; adenosine and three AMP isoforms (5′AMP, 3′AMP and 2′AMP). Hydrolysis was also observed for c-di-GMP in crude extracts. The ability to cleave c-di-AMP was further demonstrated in molecular docking simulations and *in vitro* enzyme assays for some plant phosphodiesterases previously known to degrade cyclic nucleotides. These results were accompanied by increased *t*-resveratrol levels, indicating the onset of a stress response, observed after elicitation of *Vitis vinifera* suspension cell cultures with c-di-AMP and c-di-GMP. Considering the aforementioned results and the effect of prokaryotic CDNs on animal cells, we postulate that plant cells can potentially perceive cyclic dinucleotides as pathogen-associated molecular patterns (PAMPs) inducing plant immunity response.

This work was partially supported by the National Science Centre, Poland, grant number 2022/47/B/NZ9/01088 for JM and MPB, and by the Polish Minister of Science and Higher Education as part of the Strategy of the Poznan University of Life Sciences for 2024-2026, grant number NR21/PREIDUB/JUNIOR/2024 for JM.

O-86

Crop root secretions hold promise of *Aphanomyces* root rot management in pulses

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Keywords: aphanomyces euteiches, root-rot, crop rotation, root exudates, pulses

Aphanomyces root rot (ARR), caused by *Aphanomyces euteiches*, is one of the most devastating soilborne diseases affecting pulse crops, posing a significant threat to production in both Europe and Canada. The absence of effective chemical or biological controls, coupled with the pathogen's long-term persistence in soil, increases the risk of ARR spread, thus limiting yield potential and threatening the sustainability of legume-based cropping systems. Currently, crop rotation is the only viable management strategy. However, the lengthy rotation period required, six years or more, is often impractical for growers, leading many to abandon pulse cultivation altogether. This was reflected in a 15-20% decline in acreage under pulses in Canada. We previously reported that root exudates of susceptible and partially resistant pea cultivars have distinct metabolic profiles. The presence of an array of saponins inhibited the activation of dormant oospores in soil, which could be a contributory factor in partial resistance, especially during the pre-invasion phase of the pathogen (Goyal et al., 2024). To learn more about how crop rotation works to reduce the ARR, the root exudates of common commercial non-leguminous crops used in rotation were studied. We observed a significant contrast among different crops. Some crop-root exudates favored the activation of Aphanomyces oospores, while others showed a strong inhibitory effect. The findings can form the basis of making the crop rotation more effective and smart.

This work was supported by the grants from Alberta Pulse Growers and Results Driven Agriculture Research of Alberta (Grant number, 2025F3676R).

Goyal, R.K., J. P. M. Hui, J. Ranches, R. Stefanova, A. Jones, A. H. Banskota, et al (2024). Untargeted metabolomic analysis reveals a potential role of saponins in the partial resistance of pea (Pisum sativum) against a root rot pathogen, Aphanomyces euteiches. Phytopathology. https://doi.org/10.1094/PHYTO-04-24-0151-R

O-87

Pathogen's playbook: Phytohormone production and manipulation by *Leptosphaeria maculans*

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Keywords: phytohormones, auxin, cytokinin, salicylic acid, *Leptosphaeria maculans*, *Brassica napus*, virulence

Phytohormones are small molecules that play a critical role in regulating plant life processes, including growth, development and defense responses. The fungal pathogen *Leptosphaeria maculans*, which causes blackleg disease in *Brassica napus* (oilseed rape), has evolved mechanisms to manipulate host phytohormone pathways, thereby potentially enhancing its virulence. This pathogen is capable of synthesizing a range of phytohormones, including auxins, cytokinins (CKs), and salicylic acid (SA).

Auxin production in *L. maculans* is stimulated by biosynthetic precursors tryptophan and tryptamine and involves transcriptional activation of genes such as *LmTAM1* and *LmIPDC2*. Auxin excretion by the fungus has been observed, and exogenous auxin application modulates necrotic lesion size on *B. napus*. Cytokinin biosynthesis is mediated by enzymes isopentenyltransferase (LmIPT) and adenosine kinase (LmAK). Suppression of CK biosynthesis compromises fungal fitness and virulence. CK profiles in *L. maculans* differ from those in the host plant, with free CK bases predominating in the fungal mycelium. Additionally, SA production has been detected in *L. maculans*, with orthologues of plant biosynthetic genes such as *AtICS1* identified. The fungus also exhibits an SA-sensing mechanism involving the SA-responsive gene *LmSrg1*. The interplay between SA and auxin signaling underscores the complexity of hormonal crosstalk during infection.

These findings suggest that *L. maculans* not only produces phytohormones but also utilizes them to manipulate host signaling pathways. Understanding these mechanisms provides valuable insights into the

molecular basis of fungal pathogenicity and offers potential targets for developing strategies to combat blackleg disease in oilseed rape.

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O-88

Nitric oxide/nitroxyl interplay during plant-pathogen interaction

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Keywords: nitric oxide; nitroxyl, total antioxidant capacity, *Solanum tuberosum*, *Phytophthora infestans*

Nitroxyl (HNO/NO–, also named azanone) was documented as a weak acid (pKa 11.4), suggesting that HNO, rather than the nitroxyl anion (NO–), predominates in physiological states. The chemistry of this simple, triatomic species is highly complex, and it can react with many targets, including molecular oxygen, nitric oxide, nitrite, hydroxylamine, sulfite, thiosulfate, metalloproteins, metalloporphyrins, thiols, C- and S-nitroso compounds, nitroxides, and phosphines. This reactivity makes it a perfect candidate for a signaling molecule. Recently, the endogenous production of HNO in plant tissues (*Arabidopsis thaliana* L.) was reported for the first time, suggesting a novel regulatory or signaling role in plant cells. Under physiological conditions, HNO concentrations were documented in the nanomolar range. Thus, the ubiquitous bioavailability of HNO allows it to support or compete with nitric oxide (•NO) signaling, depending on the local redox environment.

In the present study, we showed that the pathogen attack promotes not only •NO but also HNO formation in host cells. Using electrochemical microsensors measuring HNO and •NO concentration up to low nanomolar levels in real-time, we detected that inoculation of potato (*Solanum tuberosum* L.) leaves with *Phytophthora infestans* resulted in an early HNO and •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 •NO/HNO interplay during the potato – Avr/vr *P. infestans* interaction.

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■

O-89

Extracellular microRNAs: Plants' first line of defense?

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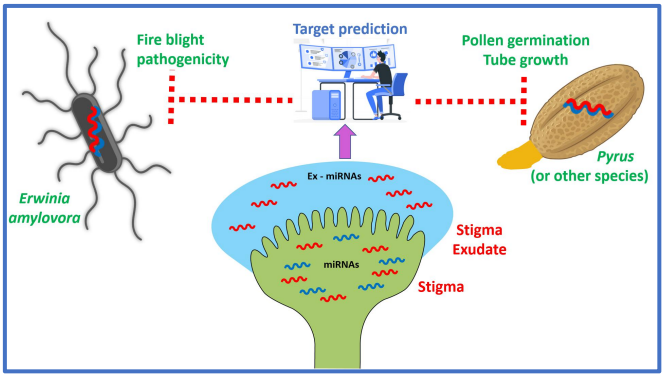
Keywords: pear, *Erwinia amylovora*, extracellular microRNAs

Extracellular RNAs (exRNAs) have been extensively studied in mammalian systems, and are associated with infectious diseases and developmental processes. In plants, exRNAs have been only scarcely studied. Yet, recent studies show that plant exRNAs can be transmitted to cells from various kingdoms, including mammals or fungi, and interfere with their transcriptional regulation. Recently, the Leshem lab discovered, for the first time, the presence of exRNAs in *Pyrus* stigma exudates, including mature microRNAs known from other *Rosaceae* species (Ambastha et al., 2023). Computed predicted microRNA targets suggest their possible involvement in stigma-pollen crosstalk, and remarkably, also identified potential targets in the genome of *Erwinia amylovora*, the causal agent of the devastating fire blight disease in *Pyrus* spp., that threatens global fruit production. These findings suggest that microRNAs could be involved in cross-kingdom host-pathogen interactions, acting as a first line of defence against bacterial infection. Our discovery could potentially lead to the development of novel bio-control strategies for controlling fire blight, based on the naturally produced microRNAs.



Ambastha V, Nevo Y, Matityhu I, Honys D, Leshem Y (2023) Discovery of microRNAs in Pyrus stigma exudates opens new research avenues in Horticulture. PNAS Nexus 2: 1–4

O-90

Dissection of the antiviral RNA silencing pathway of *Nicotiana benthamiana* by genome editing

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Keywords: RNA silencing, virus, Nicotiana benthamiana

In plants, a number of protective measures have been evolved to combat viral infections. Of these, antiviral RNA silencing has been the most thoroughly characterized. Studies relying on the extensive genetic resources of *Arabidopsis thaliana* have greatly helped clarify the details of the molecular arms race between plants and viruses. However, the use of *Arabidopsis thaliana* as a virological model plant is limited. Most importantly, this species is a non-host of many important pathogenic viruses that are responsible for enormous damage to economically important crops. In contrast, the native Australian tobacco, *Nicotiana benthamiana*, has an unparalleled susceptibility to viruses. Moreover, since it belongs to the *Solanaceae* family, it is considered an adequate model system for studying infectious diseases of extremely valuable crops such as tomatoes, potatoes, pepper and tobacco. Nevertheless, its adoption as a genuine model species has so far been hampered by the amphidiploid nature of its genome. The latest technical advances in genome editing and next-generation sequencing however, can help overcome this limitation.

Our laboratory has been employing the CRISPR/Cas9 genome editing system to mutagenize key genes of the antiviral RNA silencing pathway of *Nicotiana benthamiana*. Over the years we have created a number of mutants including those that carry mutations in the catalytic cores of antiviral RISCs (Argonautes, AGOs) and in amplifiers of RNA silencing (RNA-dependent RNA polymerases, RDRs). Relying on this inventory of mutants, we demonstrated that sequential hierarchical actions of multiple AGOs are needed to build effective antiviral resistance *in planta*. Furthermore, our results suggest that in addition to *RDR1* deficiency, the loss of several key genes associated with RNA silencing may also contribute to *Nicotiana benthamiana*'s hypersusceptibility to viruses. In conclusion, the rapidly evolving field of genome editing helps *Nicotiana benthamiana* to rise to the level of a *bona fide* model species, which, in addition to its basic research value, is also of great practical importance, as this plant has become a popular chassis for molecular pharming in recent years.

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O-91

Investigation of VSR coding capacity of fruit tree infecting viruses

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Keywords: virus, RNAi, viral silencing suppressor

Fruit trees are constantly exposed to the infection of viruses. Persisting in the orchards for decades, they could become infected with several unrelated viruses. The host RNA interference (RNAi) based defence mechanism is activated during a viral infection. To evade this mechanism, viruses have developed proteins that function as viral suppressors of RNAi (VSRs). Plant defence reactions can play a key role in the severity of the symptoms. As they are affected by the efficiency of the VSRs, the severity of the symptoms can be directly connected to the presence and activity of VSRs.

Recently, we have characterised viromes of apple, sweet cherry and sour cherry trees using high-throughput sequencing (HTS) [1,2] and identified widespread infection of the apple trees with apple luteovirus 1, citrus concave gum, virus cherry virus A and Prunus virus F. Although these viruses were only recently described, they have not yet been characterised molecularly. Our study aimed to check the possibility of whether any of the proteins encoded by them pos sess VSR activity, and if so, how effectively they can inhibit the plant's defense system both locally and systemically. We aimed to compare the activity of possible VSRs, both alone and in combinations, to mimic the frequent coinfections observed during our surveys.

For this, an *Agrobacterium*-mediated transient gene expression assay was performed, using GFP as an inducer of the silencing. The intensity of the silencing was monitored both locally and systemically.

Based on our results, we found VSR activity in several cases, which could explain why co-infections in the fruit trees at the orchard may lead to increased symptom severity.

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O-92

Genotypic screening of *Capsicum* germplasm for disease resistance using fluidigm SNP markers

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Keywords: Capsicum, disease resistance, Fluidigm genotyping, SNP markers

Pepper plants (*Capsicum* spp.) are widely cultivated for their economic and nutritional value, but they are susceptible to various diseases that significantly impact yield and quality. This research investigated a wide range of *Capsicum* accessions (5658), collected from different species and geographical regions, and stored at the National Agrobiodiversity Center, Genebank. Using 19 SNP markers and the Fluidigm genotyping system, we assessed these accessions for resistance against eight common pepper diseases. The findings revealed accessions with resistance to specific diseases, as well as those showing resistance to multiple diseases, such as bacterial spot, anthracnose, powdery mildew, phytophthora root rot, and potyvirus. *C. chacoense* accessions were identified as highly resistant to bacterial spot, anthracnose, powdery mildew, and phytophthora root rot, highlighting the strong natural defense mechanisms of wild *Capsicum* species and their potential as sources of resistance for breeding programs. Additionally, *C. baccatum* species were found to be promising candidates for resistance to key pepper diseases. Overall, disease-resistant germplasm from different *Capsicum* species was identified, with accessions from regions such as Argentina, Bolivia, and the United Kingdom consistently showing resistance. This suggests that disease-resistant traits are widely distributed across diverse environments. Furthermore, ten accessions were selected based on their multi-disease resistance, including resistance to CMV, phytophthora root rot, potyviruses, and TSWV, from regions like Hungary, Peru, the United States, and the Netherlands. This comprehensive study offers valuable insights into disease resistance mechanisms in *Capsicum*, which is essential for promoting sustainable agricultural practices and enhancing crop improvement through breeding strategies.

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O-93

RETINOBLASTOMA-RELATED has both canonical and non-canonical regulatory functions during thermo-morphogenic responses in *Arabidopsis* seedlings

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Warm temperatures accelerate plant growth, but the underlying molecular mechanism is not fully understood. Here, we show that increasing the temperature from 22°C to 28°C rapidly activates proliferation in the apical shoot and root meristems of wild-type Arabidopsis seedlings. We found that one of the central regulators of cell

proliferation, the cell cycle inhibitor RETINOBLASTOMA-RELATED (RBR), is suppressed by warm temperatures. RBR became hyper-phosphorylated at a conserved CYCLIN-DEPENDENT KINASE (CDK) site in young seedlings growing at 28°C, in parallel with the stimulation of the expressions of the regulatory CYCLIN D/A subunits of CDK(s). Interestingly, while under warm temperatures ectopic RBR slowed down the acceleration of cell proliferation, it triggered elongation growth of post-mitotic cells in the hypocotyl. In agreement, the central regulatory genes of thermomorphogenic response, including *PIF4* and *PIF7*, as well as their downstream auxin biosynthetic *YUCCA* genes (*YUC1-2* and *YUC8-9*) were all up-regulated in the ectopic RBR expressing line but down-regulated in a mutant line with reduced RBR level. We suggest that RBR has both canonical and non-canonical functions under warm temperatures to control proliferative and elongation growth, respectively.

O-94

Pectin chemistry effects non-uniform cell growth during mesophyll morphogenesis

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Keywords: Mesophyll, pectin, micro-CT

Plant leaves exhibit complex structural organisation that facilitates crucial biophysical processes such as photosynthesis and respiration. The site for photosynthetic carbon fixation is the internal tissue, the mesophyll, whose 3D geometry is highly complex and comprises a network of air spaces. Structural traits such as cell shape and volume, intercellular connectivity of air spaces, the expanse of the internal cell surface facing air spaces, and the porosity of the mesophyll tissue affect mesophyll conductance and net photosynthetic capacity. Thus, mesophyll cell morphogenesis and the formation of intercellular spaces are two major processes influencing the functional architecture of leaves. Non-uniform and anisotropic cell growth and cell-cell detachment involved in the transformation of dense embryonic tissue into a complex aerenchymatic one is controlled by cell wall polysaccharides. The differential distribution of cell wall polysaccharides such as cellulose, xyloglucans, pectin and other homogalacturonans influence cell wall properties such as elasticity and expansibility as well as cell-cell adherence at the middle lamella. Here we use *Arabidopsis thaliana* wildtype and mutants with altered pectin methyl esterification to elucidate how cell wall polysaccharide composition correlates with tissue morphogenesis and how altered cell wall properties affect leaf anatomy. Confocal laser scanning microscopy and synchrotron-based X-ray microcomputed tomography (micro-CT) techniques are employed to characterize mesophyll 3D architecture and dynamic changes through cell morphogenesis. Mapping cell wall polysaccharides during development through immunohistochemistry revealed enrichment of lowly methyl esterified pectin in cell wall segments that maintain cell adhesion during air space formation. Genetically altered mutants with increased levels of highly methyl esterified pectin display enhanced cell wall expansion resulting in bigger cells. The dynamic changes in pectin chemistry play an important role in dictating the growth of mesophyll cells, their morphogenesis and cellular adhesion thereby affecting tissue porosity.

O-95

ERAD-mediated maturation of the regulatory protein of plant meristematic cells CLAVATA 3 emerged during evolution from algae to higher plants

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Keywords: CLAVATA3, plant meristems, ERAD process

The indefinite growth capacity and organogenesis in plants are guaranteed by meristems, tissues highly regulated by intercellular signalling pathways where cells are in continuous division and differentiation. In the shoot apical meristem of *Arabidopsis thaliana*, the differentiation-promoting peptide derived from the proteolytic maturation of the protein CLAVATA3(CLV3) establishes an autoregulatory negative-feedback loop that modulates the expression of the stem cell-promoting transcription factor WUSCHEL [1]. The shoot

apical meristem remains alive for the entire plant life, balancing the continuous loss of daughter cells during organogenesis. Published studies on the maturation process of the secretory protein CLV3 seem to suggest a mechanism of proteolysis mediated by proteases secreted in the apoplast, that lead to the formation of the dodecapeptide, capable of acting as a ligand of the CLV1/CLV2 receptor complex. Recent studies in tobacco have suggested that the CLV3 maturation process may occur not in the apoplast but through the intracellular ERAD (Endoplasmic Reticulum Associated Degradation), a cellular quality control system naturally used by cells to degrade misfolded proteins [2]. To test this hypothesis, we propose the expression of the fusion protein CLV3-GFP in *Arabidopsis thaliana* and *Nicotiana tabacum* plants and protoplasts, monitoring its localisation using multiple biomolecular and imaging techniques. Additionally, a CLV3-YFP transgenic strain of *Chlamydomonas reinhardtii* has been developed, to follow the maturation process of this protein in an organism without meristem organisation and endogenous *CLV3* expression. To further investigate whether the protein maturation process is linked to the ERAD mechanism, the potential role of ubiquitination has been studied, as it is a key initiator of proteasome-mediated degradation: the most probable ubiquitinated lysine residues in the CLV3 protein sequence were mutated to block ubiquitin-ligase activity, thereby potentially altering the maturation of CLV3. The results suggest an alternative maturation pathway for CLV3, distinct from the canonical secretion: various genetic and biochemical strategies were employed to shed light on this process, which appears to follow different routes in different biosystems.

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O-96

The role of phytochromes A and B in the regulation of fruit metabolism and chloroplast ultrastructure in tomato leaves under different ratios of red and far-red light

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Keywords: *Solanum lycopersicum*, phytochromes, ratios of red and far-red light

The spectral composition of light regulates tomato (*Solanum lycopersicum*) metabolism via phytochromes, which coordinate carbohydrate metabolism, antioxidant activity, and structural characteristics of chloroplasts. In this work, wild-type and mutant lines (*phya, phyb1, phyb2, phyab2, and phyab1b2*) were studied at different ratios of red and far-red light (RL:FRL).

At RL:FRL = 1:2, the *phyb2* mutant presented a 17-fold decrease in sucrose content in fruits, which was associated with the suppression of glycolysis. Under these conditions, the ATP level decreases, and phosphofructokinase activity decreases, which limits carbon flow into the Calvin–Benson cycle. In contrast, at RL:FRL = 2:1 in *phyb2*, the content of ribulose-1,5-bisphosphate increased 41-fold, and that of ascorbic acid increased 18-fold, indicating activation of the Calvin–Benson cycle and increased antioxidant protection due to ascorbate peroxidase.

Secondary metabolism also depends on the RL:FRL ratio. At RL:FRL = 1:1 in the *phyab2* mutant, *PAL* gene expression increased 4.8-fold, which was accompanied by an increase in the chlorogenic acid level of 63%, indicating an increase in the phenylpropanoid pathway. In the *phya* mutant, at RL:FRL = 2:1, flavonoid accumulation decreased, which was associated with the suppression of *FLS*.

Ultrastructural analysis revealed that in *phya*, at elevated FRL levels, the lamellar lumen decreased, and chloroplasts became agranal, which impaired their photosynthetic efficiency. In *phyb2*, at RL:FRL = 1:1, starch

accumulation was observed due to an imbalance between CO_2 fixation and assimilate transport. Even more pronounced starch accumulation was observed in the *phyab1b2* mutant.

These results demonstrate the key role of phytochromes in regulating chloroplast metabolism and ultrastructure. Optimization of the spectral composition of light provides opportunities for managing the nutritional value and shelf-life of tomatoes.

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O-97

How parental factors shape the plant embryo

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Breaking symmetry by asymmetric cell divisions is essential for establishing different cell identities in multi-cellular development. Cell-cell signaling by receptor kinase/MAP kinase signaling pathways seems to be a reoccurring mechanism in polarizing cells and establishing different cell identities in the daughter cell of asymmetric cell divisions in land plants.

We are using the early *Arabidopsis* embryo as system to study initial events in cell polarization by the ERECTA-YODA signaling pathway – a prototype receptor kinase/MAP kinase signaling pathway.

Embryogenesis resembles a linear system of developmental progression with successive asymmetric cell divisions that produce the primary tissue types of the plant seedling and establish the stem cell niches of the shoot and root. By comparing the function of the ERECTA-YODA pathway in the embryo with its role in planar patterning of the leaf epidermis, we identified principal functions of a core pathway and context-specific modifications.

New data sheds light on the impact of polar YODA activation on early embryonic patterning and how both parents influence the early embryonic development. We will highlight the mechanism and evolution of distinct modes of YDA activation in the zygote on a molecular and structural level. We furthermore discuss possible benefits of different modes of YDA activation and their distinct parent-of-origin effects.

O-98

Somatic embryogenesis is an ancestral reproductive strategy in plants

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Keywords: somatic embryogenesis, phylotranscriptomics, *Vitis vinifera*

Somatic embryogenesis is a process in which any somatic plant cell dedifferentiates into an embryo-competent state and turns on its embryogenic program. This process is independent of any reproductive tissues or fertilization, and represents the strongest demonstration of plant cell totipotency. Specific plant growth regulators or the application of stress conditions are often used to stimulate embryogenic competence in somatic cells cultivated *in vitro*. Because it is rarely observed in nature, the significance of somatic embryogenesis has been underestimated for many years. In our research, we have developed an efficient system for somatic embryogenesis in grapevine (*Vitis vinifera* L.) from which the transcriptomes of 12 consecutive developmental stages were obtained. We observed that essentially all genes (99.56% of the annotated *V. vinifera* genes) were transcribed at some point along the developmental trajectory of somatic embryogenesis. To determine whether somatic embryogenesis correlates with the evolutionary trajectory of the plant lineage, we linked the transcriptome (expression levels) of 12 developmental stages to the evolutionary age of *V. vinifera* genes using a phylostratigraphic approach. By combining the evolutionary age of grapevine genes with their expression levels during somatic embryogenesis, we found a strongly supported hourglass-shaped developmental trajectory. In contrast to zygotic embryogenesis in Arabidopsis, where the torpedo stage is the most evolutionarily inert (expressing the evolutionary oldest genes; the phylotypic stage), in grapevine somatic embryogenesis we identified the heart stage as the phylotypic stage. In conclusion, our results revealed that somatic embryogenesis is a full-fledged developmental process that utilizes essentially all available protein-coding genetic information. Moreover,

due to phylotypic stage determined in the mid-stage of somatic embryogenesis, we have shown a better evolutionary system-level analogy between animal development and plant somatic embryogenesis than zygotic embryogenesis, indicating that somatic embryogenesis is likely a primordial embryogenic program in plants. Since somatic embryogenesis can be induced by stress in most plant species, it would be interesting to determine whether this ancestral pathway of embryogenesis is also an atavistic trait that plants have retained in their evolutionary memory.

O-99

Intracellular glass fragility distinguishes the desiccation response of embryonic axes from recalcitrant and orthodox seeds

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Keywords: anhydrobiosis, desiccation, seeds

Seed tolerance to desiccation and freezing determines the requirements for long-term plant ex-situ conservation in germplasm banks. Desiccation- and freezing-tolerant (i.e., orthodox) seeds are banked at 15% RH and -20 °C, which are lethal to sensitive (i.e., recalcitrant) seeds that require tissue cryopreservation. These contrasting responses are postulated to depend on the ability of orthodox seeds to form stronger (less fragile) intracellular glasses than recalcitrant seeds. Here, we assessed this hypothesis using dynamic mechanical analysis to compare the viscoelastic properties of intracellular glasses in the embryonic axes (EAs) of recalcitrant (*Aesculus hippocastanum*, *Aesculus indica*) and orthodox seeds, either long- (*Pisum sativum*, pea) or short-lived (*Glycine max*, soybean). Across water contents (WCs), all species showed α-relaxations, equivalent to glass transitions, whose size linearly increased with WC in *A. hippocastanum* and *P. sativum*, whereas *A. indica* (more sensitive to cryopreservation than A. hippocastanum) and G. max revealed anomalous plasticisation effects. Plotting the onset and peak temperatures of the α-relaxations against WCs did not differentiate between the four species. Nonetheless, a glass fragility index, accounting for the temperature ranges associated to the α-relaxations, discriminated between seed orthodoxy and recalcitrance in response to desiccation. Under hydrated conditions conducive to physiological activity (~0.25-0.30 g water g^{-1} dry weight [DW]), the fragility of intracellular glasses was similar between recalcitrant and long-lived orthodox seeds, while *G. max* formed a stronger intracellular glass. Drying increased the glass fragility of both orthodox and recalcitrant-seeded species. However, below WCs critical to cryopreservation of recalcitrant seeds (~0.20-0.25 g water g^{-1} DW), their EAs died while forming more fragile intracellular glasses, compared to the EAs of orthodox seeds that survived and formed relatively stronger intracellular glasses. Overall, as found in other anhydrobiotes, we provide evidence that differences in the structural properties of intracellular glasses are key to plant survival in the dry state.

O-100

Insights into the sex determination mechanisms and inheritance of dioecy and monoecy traits in *Cannabis sativa* (hemp)

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Cannabis sativa, commonly known as hemp, belongs to the Cannabaceae family and has some of the oldest known plant heteromorphic sex chromosomes. Hemp is a multipurpose and versatile crop with diverse applications across various industrial sectors. Naturally, hemp is a dioecious species where male and female flowers are present on different plants. However, monoecious cultivars with male and female flowers on the same plants have also been bred for industrial purposes. Several studies have shown XY chromosomes control sex determination in hemp. However, the inheritance of dioecy and monoecy traits remains elusive. In the current study, we addressed this question using F1 populations resulting from crosses between dioecious and monoecious cultivars. Our results showed that dioecy was a dominant trait over monoecy in several crosses made, as F1 populations consisted mainly of dioecious male and female individuals with only few monoecious individuals. Further, in F1 progenies, we did not observe any deviation from the classical sex ratio of 1:1, further supporting the dioecy of the plants. Thus, our study describes the dioecy as a dominant trait linked with the X chromosome with some level of plasticity. These findings can prove helpful in guiding hemp breeding programs.

O-101

Interactive effects of silver nanoparticles and microplastics on oxidative stress in *Allium cepa* roots

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Keywords: microplastics, onion, oxidative stress, silver nanoparticles

Silver nanoparticles (AgNPs) have antimicrobial and antifungal properties, but pose a risk to the environment. Microplastics (MPs), also a persistent pollutant, can adsorb AgNPs on their surface and thus affect their transport and bioavailability. Both pollutants accumulate in plants either by adhering to the root surface or by entering the shoots through the vascular tissue. The interaction between MPs and AgNPs may alter their toxicity, raising concerns about their combined effects on plants. In this study, *Allium cepa* roots were exposed to AgNPs with different coatings (polyvinylpyrrolidone (PVP) and cetyltrimethylammonium bromide (CTAB)) and MPs made of polystyrene (PS-MPs) or polymethyl methacrylate (PMMA-MPs) at different concentrations. The effect of combined exposure of each type of MPs with each type of AgNPs was also investigated. The uptake of AgNPs and MPs was measured together with markers of oxidative stress and the activity of antioxidant enzymes. Silver uptake data showed that AgNP-CTAB penetrated root cells more efficiently than AgNP-PVP when used alone. However, when combined treatments were used, the trend was reversed, with MPs significantly reducing AgNP-CTAB uptake. MPs uptake results showed that both types of MPs penetrated onion root cells and that as treatment concentration increased, MPs content also increased. In the combined treatments, AgNP-CTAB increased the uptake of both types of MPs, while AgNP-PVP had no effect on their uptake into onion roots. Hydrogen peroxide content increased in all treatments with both types of AgNPs, individually and in combination, while it remained unchanged with MPs alone. Neither lipid peroxidation nor protein carbonyl content was affected by any treatment. Catalase activity was highest with the individual AgNPs treatments, with AgNP-CTAB exhibiting higher toxicity, while treatments with MPs alone or in combination with AgNPs had no effect. A similar pattern was observed for ascorbate peroxidase, with AgNP-PVP having the highest toxicity, while pyrogallol peroxidase activity was highest in the AgNP-PVP treatments, both alone and in combination with both types of MPs. Superoxide dismutase activity increased with all treatments, most strongly with AgNP-PVP. The results show that the effect of the combined treatments depends on both the AgNPs coating and the MPs polymer.

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O-102

Effect of silver nanoparticles (AgNPs) in apricot seedlings submitted to mild salinity

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Keywords: apricot, physiology, metabolomics, salinity, silver nanoparticles

While it has been described that nanoparticles can enhance plant growth, a high concentration induces stress/toxicity. Silver nanoparticles (AgNPs) have gained importance in agriculture in recent years thanks to their unique characteristics, affecting plants at different levels (morphology, physiology and biochemistry). Nevertheless, plant responses to AgNPs may differ from species to species depending on concentration, application mode, exposure time and growing conditions.

In this work, *in vitro* apricot plants were propagated in presence (AgIV) or absence of AgNPs. Then, plants were acclimmatized to *ex* vitro conditions and after eight weeks of growing under greenhouse conditions, half of those micropropagated plants in absence of AgNPs were sprayed with a 200 mg/L AgNPs+Tween 20 solution (Ag) once per week for three weeks. Starting the last week of AgNPs spraying, half of the plants were watered twice per week with a NaCl solution for 6 weeks (50mM NaCl week 1-4; 100mM NaCl week 5-6). This results in six different treatments: untreated plants, Control; untreated+NaCl, C_NaCl; Ag; Ag+NaCl; AgIV and AgIV+NaCl.

Salinity had no deleterious effect on plant grow, which may be related to the adaptation of this apricot variety to grow in calcareous soils. At the end of the experiment an increase in lipid peroxidation (an oxidative stress marker)

was observed in Ag plants and in both AgIV plants (±NaCl). Ion homeostasis was altered by the different treatments, with Na^+ and Cl^- content increasing by the salinity and/or the AgNPs treatments. The photosynthesis process was slightly affected, with a decrease in photochemical quenching parameters [y(PSII) and qP] and an increase in the non-photochemical quenching parameters, the latter indicating a safer dissipation of excess energy by heat, induced by salinity and/or the AgNPs treatments.

At metabolomic level, whereas salt treated plants did not show significant altered metabolism, AgNPs plants displayed induced steroid metabolism, by means of an stimulation of brassinosteroids, and flavonoid byosintheis pathways.

Taking together, our results suggest that an AgNPs-induced oxidative signalling triggers apricot plant responses at different levels.

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O-103

Integration of chitosan nanoparticles and cyanobacteria biomass in agricultural applications to enhance cereal crop productivity

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Keywords: cyanobacteria biomass, chitosan nanoparticle, nitrogen supply, maize, wheat, barley

The integration of agriculture with other scientific fields, such as the use of chitosan and cyanobacteria, has the potential to significantly optimize fertilizer efficiency and improve soil health. The combination of the nutrient delivery and metabolic enhancement properties of chitosan with the nitrogen fixation ability of cyanobacteria within the plant offers a comprehensive approach to boost crop growth and promote sustainable soil management. Exploration of the use of chitosan nanoparticles and cyanobacteria biomass in agriculture is gaining increasing attention due to its potential to improve sustainability and crop performance [1]. However, the combined application of these two fields remains largely unexplored, necessitating further investigation into their synergistic potential alongside nitrogen fertilizers. This research seeks to investigate the effects of the integration of chitosan nanoparticles and cyanobacteria biomass – independently or in combination – with recommended and reduced nitrogen fertilizer on the productivity of cereal crops (such as maize, winter wheat, and winter barley) under field conditions. The results obtained demonstrate that the integration of chitosan nanoparticles and cyanobacteria biomass leads to a significant enhancement in crop yield and chlorophyll content, while concurrently exerting a positive influence on a range of plant growth parameters. This combined application has been shown to engender an improvement in overall plant health and productivity, whilst concomitantly enhancing physiological traits that contribute to more robust and efficient crop development. The strategic integration of agricultural practices with advanced scientific approaches, such as the combination of chitosan nanoparticles and cyanobacteria (or microalgae) biomass, can effectively extend the benefits of fertilizers while minimizing their limitations. This multidisciplinary approach not only optimizes nutrient use, but also enhances plant growth and productivity, paving the way for more sustainable and efficient agricultural systems.

[1] Molnár, Z., Lamnganbi, M., Solomon, W., & Janda, T. (2023). Agrosystems, Geosciences & Environment, 6, e20428. https://doi.org/10.1002/agg2.20428

O-104

Enhanced cellular internalization: A predominant bactericidal mechanism of biogenic nanoparticles over chemical counterparts

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The biogenic synthesis of silver nanoparticles (AgNPs) has emerged as a promising approach for enhanced antimicrobial activity, emphasizing the need for efficient biological candidates capable of capping and shaping nanoparticles through their secondary metabolites. This study explores the enhanced bactericidal potential of AgNPs synthesized using the cell-free filtrate of *Trichoderma viride* (MTCC 5661) compared to chemically synthesized citrate-stabilized AgNPs. The nanoparticles were characterized using UV–visible spectroscopy, zetasizer, TEM, GC–MS, EDAX, XRD, and FTIR, confirming their monodispersity, spherical shape, and size range of

10–20 nm, with metabolite-functionalized surfaces. Antimicrobial efficacy was evaluated against *Shigella sonnei*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* through growth inhibition curves and CFU assays. Notably, biogenic AgNPs exhibited higher cellular internalization, leading to increased reactive oxygen species (ROS) generation and severe membrane damage. This oxidative stress amplified particle uptake, triggering multiple bactericidal pathways and rapid pathogen disintegration, as demonstrated by fluorescein diacetate/propidium iodide dual staining and TEM analysis. The findings highlight the superior antimicrobial potency of biologically synthesized silver nanoarchitectures over their chemical counterparts, underscoring their potential for advanced therapeutic applications.

O-105

The impact of nitric oxide signal on the intracellular iron distribution

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Keywords: S-nitrosoglutathione reductase, X-ray fluorescence microscopy, particle-induced X-ray fluorescence

The photosynthetic apparatus requires the incorporation of a significant amount of transition metal cofactors, among others Fe containing FeS clusters and heme groups. Despite the importance of Fe loading into chloroplasts, transition metal allocation in cells is not a unidirectional process, since eukaryotic cells perform a dynamic structural reorganisation in order to adapt to the challenges of the environment. Nevertheless, the control over Fe allocation to the cell compartment in the mesophyll remained poorly known. Since nitric oxide (NO) has been found to be an important factor in the coordination of the root Fe uptake and homeostasis, its role in the mesophyll cell Fe homeostasis is also proposed. Cryosectioned mesophyll cells of early senescent *Arabidopsis thaliana* lines affected in the NO homeostasis by defects of NO biosynthesis (*noa1*) and affected in the S-nitrosoglutathione reductase enzyme (GSNOR, deficient and overexpressing) were to particle induced X-ray emission (μPIXE), low energy X-ray fluorescence microscopy (LEXRF), and high energy (HE) XRF microscopy. The HEXRF dataset is accessible at Solti et al. (2025). K-means clustering of the Fe signal distribution approved that perturbed NO signaling resulted in a lowered plastidial Fe allocation in the early senescent stage that underlines impact and the pro-senescence property of the NO signaling nature on the plastidial Fe accumulation.

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Solti Á & al. (2025). ESRF. doi.org/10.15151/ESRF-ES-790328283 [Dataset].

O-106

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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Keywords: transparent soil, Zn transport, roots, xylem, phloem

Zinc is an essential element for plant development. However, its distribution in soil is heterogeneous, affecting factors such as root growth efficiency. The control over Zn distribution in and between organs exists; however, the mechanisms that govern this process are unknown. We use tools like transparent soil, a hydrogel-based medium that mimics soil properties and allows us

to heterogeneously distribute Zn (Zn sufficient and deficient parts in one pot) and track root growth. We demonstrated that the expression of tobacco ZIPs (Zn uptake), HMAs (Zn translocation to shoot) and NASs (Zn vacuolar storage and phloem distribution) depends on the total accessibility of the root system to Zn, rather than local Zn levels. Further we confirmed for the first time, using μXRF at Polish Synchrotron SOLARIS, (beamline POLYX), 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 of the same plant. This surprising finding suggests the existence of previously unknown Zn homeostasis mechanisms in roots, likely involving an active and 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 root or shoot regions, independent of Zn concentration in the surrounding medium. We further identified Zn relocation sites where Zn is potentially transferred from xylem to phloem to be delivered to the latera root growing in Zn deficient part of the medium. Moreover, we showed that different Zn distribution scenarios affected Zn content and distribution in shoots. We propose that this work provides new insights into plant Zn homeostasis, particularly regarding Zn translocation between tissues and organs, and may inform future agricultural practices to address Zn deficiency.

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O-107

Genetic control of the leaf ionome in pearl millet and correlation with root and agromorphological traits

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Keywords: Pearl millet, leaves, ionomics, GWAS, nutrient use efficiency, root, cereal

Pearl millet (*Pennisetum glaucum*) thrives in arid and nutrient-poor environments, establishing its role as a crucial cereal crop for food security in sub-Saharan Africa. Despite its remarkable adaptability, its yield remains below genetic potential, primarily due to limited water and nutrient availability. In this study, we conducted ionomic profiling and genome-wide association studies (GWAS) under field conditions across two growing seasons to unravel the genetic basis of nutrient acquisition in pearl millet. Soil ion content analyses revealed significant differences in nutrient distribution between field sites, while certain ions, such as phosphorus (P) and zinc (Zn), consistently displayed stratified accumulation patterns across years, suggesting stable depth-dependent trends.

Evaluation of a genetically diverse panel of inbred lines revealed substantial variation in leaf ion concentrations, with high heritability estimates. Links between leaf ion content and root or agromorphological traits showed how both genetics and the environment influence nutrient accumulation in leaves. These results also suggest that plants may need to balance different strategies for taking up nutrients. GWAS identified genomic regions associated with leaf ion concentrations, and the integration of genetic and gene expression data facilitated the identification of candidate genes implicated in ion transport and homeostasis.

Our findings provide valuable insights into the genetic regulation of nutrient acquisition in pearl millet, offering potential targets for breeding nutrient-efficient and climate-resilient varieties. This study underscores the importance of integrating genetic, physiological, and root architectural traits to enhance agricultural productivity and sustainability in resource-constrained environments.

O-108

Regulation effect of PH domain containing proteins on Mn transport and distribution

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Keywords: manganese transport, NRAMP, PH domain proteins, LEXRF, PIXE

Manganese (Mn) is essential for various plant processes, including lignin biosynthesis, cell wall formation, and enzyme activities, among others the function of the water splitting complex in photosystem II of the thylakoid membranes of chloroplasts. Although Mn homeostasis plays a pivotal role in plant cells, the regulation of Mn distribution at the cellular level is still poorly understood. Mn transporters in the plant membranes, among others NATURAL RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN 1 (NRAMP1) channel the Mn content of the cell towards the site of incorporation to enzyme proteins. Localization of NRAMP1 is regulated by PH DOMAIN CONTAINING 1 (PH1), determining the trafficking of NRAMP1. Unlike PH1, the role of PH2 in Mn transport remained unclear. Applying *Arabidopsis thaliana ph1ph2* double mutant lacking functional PH1 and PH2 proteins, we aimed to explore whether PH2 influences NRAMP1 localization and, consequently, Mn allocation in plant cells, and whether PH proteins are responsible for pleiotropic effects in Mn distribution. On cryosectioned, lyophilized samples we employed particle induced X-ray fluorescence microscopy (μPIXE) and low-energy X-ray fluorescence (LEXRF) microspectroscopy to map Mn distribution at tissue and cell levels, respectivaly. Comparing wild type and *ph1ph2* lines grown under Mn containing and Mn deprived conditions, *ph1ph2* line indicated a perturbed plastidial Mn accumulation resulted in a failure to respond the altered Mn nutrition. Along with the altered Mn distribution, we also observed minor shifts in the Fe distribution within the cells, with a noticeable shift towards the plastids.

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O-109

Mapping light-harvesting function, photoprotection responses, and thylakoid stacking efficiency in the Photosystem II antenna system of plants

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Keywords: photosynthesis, non-photochemical quenching, photoprotection, LHCII, monomeric LHCs, thylakoids

Optimal photosynthetic performance in plants requires an alignment between the level of chlorophyll excited states from light harvesting and the electron transport rate from water to carbon dioxide. Key mechanisms include fast-activated responses that dissipate excess energy as heat, collectively known as Non-Photochemical Quenching (NPQ), which minimizes the formation of

reactive excited states and prevents photoinhibition. Moreover, dynamic changes in the thylakoid macrostructure have been recognized to play a crucial role in fine-tuning photosynthetic electron transfer.

Light harvesting and its regulation involve a complex array of LHC pigment-protein complexes. However, the specific roles of each gene product in the stacking of grana and their contributions to light harvesting and photoprotective functions remain inadequately understood. In this study, we dissected the PSII antenna system by employing genome editing to target subsets of *LHCB* genes, and report on the characterization of *Arabidopsis* mutants missing specific PSII antenna components: the trimeric LHCII (*koLHCII*), the monomeric LHC (*NoM*), or both (*koLhcb*).

Electron microscopy analysis revealed that the complete absence of the PSII antenna system halted grana formation entirely, while the removal of specific Lhcb subgroups led to a 50% reduction in thylakoid stacking. Notably, Lhcb5 alone partially restored the stacking, whereas the expression of Lhcb2 produced grana with a significantly larger diameter, which disintegrated upon light-dependent phosphorylation. We further examined the NPQ mechanism and the overall resistance to photoinhibition under excess irradiation to pinpoint the specific site(s) of photoprotective responses. Our findings showed that NPQ activity was present in all genotypes, indicating that each PSII pigment-binding protein contributes to the overall quenching response. While trimeric LHCII accounted for the most significant contribution to NPQ, analysis of mutants with similar LHC content and stacked thylakoid membranes revealed that monomeric Lhcbs had the highest photoprotective activity under excess light conditions. This analysis provides genetic evidence for the functional link between PSII antennae and thylakoid stacking and demonstrates that individual LHC proteins are preferentially suited for roles in membrane cohesion, thermal energy dissipation, or photoprotection in high light conditions.

O-110

Importance of EGY2 protease for the proper functioning of chloroplasts under high-light stress

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Keywords: photosynthesis, chloroplasts, intramembrane proteases

Egy2 is an intramembrane chloroplast protease belonging to the S2P family. Intramembrane proteases play a key role in releasing membrane-anchored transcription factors through proteolytic cleavage, ultimately influencing gene expression. To date, no substrates for the Egy2 protease have been identified, however, it is known that its absence in the thylakoid membranes of *A. thaliana* is associated, among other things, with increased sensitivity of plants to photoinhibition. Our research sheds light on the significance of Egy2 for the proper functioning of the *A.thaliana* photosynthetic apparatus under short-term high-light stress. The *A. thaliana* mutants showed significant changes in the stoichiometry of chloroplast-encoded photosystem II proteins. The accumulation level of PsbA protein was increased, while the pools of the PsbC and PsbD proteins were significantly reduced. The observed changes in the stoichiometry of photosystem II core polypeptides correlate with reduced maximum quantum efficiency of photosystem II and increased non-photochemical quenching of light energy. The likely substrate for the Egy2 protease is the pTAC16 protein. Our research showed the physical interaction between Egy2 and pTAC16 and the overaccumulation of pTAC16 in the thylakoid membranes of *egy2* mutants under high-light stress conditions. These results suggest the involvement of Egy2 protease in response to high-light stress by the release of pTAC16 from the thylakoid membranes, which ultimately leads to changes in the accumulation level of PsbA, PsbC, and PsbD proteins and alterations in the stoichiometry of photosystem II complexes.

O-111

Optimising photosynthetic efficiency *in silico* through species-specific rubisco catalysis and improved protein allocation

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Keywords: photosynthesis, modelling, food security

Modelling crops *in silico* can identify bottlenecks in photosynthetic metabolism that limit the realisation of greater sustainable crop yields. To predict routes to improved theoretical crop yields, resource investment among enzymes of the Calvin-Benson-Bassham cycle (CBBc) can be optimised using targeted alterations and engineered to improve photosynthetic efficiency. Ribulose-1,6-bisphosphate carboxylase/oxygenase (Rubisco) is responsible for carbon fixation in photosynthetic organisms and is the most abundant protein on Earth. The rate of carbon fixation by Rubisco depends both on the enzyme's amount and catalytic properties. The e-Photosynthesis model represents changes in CBBc metabolite concentrations using a system of ordinary differential equations (ODEs). This is solved numerically to calculate carbon assimilation on the basis of fixed total protein investment in CBBc enzymes. Temperature dependencies of Rubisco catalysis were added to e-Photosynthesis and gas exchange data used to parameterise the model for rice. The model was then used to predict the distribution of protein between all of the CBBc enzymes that would maximise photosynthetic CO_2 uptake. The model predicted which specific CBBc enzymes would increase in association with optimisation of CO_2 assimilation. From this, strategies overexpressing target sets of CBBc enzymes were proposed for engineering improved photosynthesis across a wide range of environmental conditions – including limited diffusion under water deficit and future elevated [CO_2] under climate change. Although predicting more modest improvements than total protein redistribution, our strategies demonstrated realistic potential for photosynthetic improvement that could be achieved by DNA editing and boost rice production under global change.

O-112

Beyond the leaves: Unravelling the role of chloroplasts in the plant's stem

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Keywords: stem photosynthesis, root biomass, tomato, microscopic analysis, photochemical processes

Leaves are the crucial organs of most plants, specialized in the absorption of light energy in the PhAR (photosynthetically active radiation) range, necessary for photosynthesis and, consequently, for the development and functioning of the organism. Intensive absorption of light quanta occurs in chloroplasts - organelles containing PSI and PSII photosystems equipped with chlorophyll. Chloroplast-containing cells are also located in other plant tissues, e.g., in the vascular tissues of the stem. In most plants, a relatively small number of stomata in the stems, along with several tissues surrounding the chloroplast-containing cells and small air spaces inside these tissues, significantly impede the diffusion of carbon dioxide (CO_2), this, in turn, limits the photochemical processes and photosynthesis. Therefore, the question arises: What is the role of these tissues in justifying the energy costs associated with the presence of "green" cells in the stems?

The presented research aimed to analyze the role of chloroplasts in the stem cells of tomato (*Solanum lycopersicum* L.) plants with unshaded (control) and

partially darkened stems, and their relationship to the development of the root system as well as the aboveground parts of the plants. The study included biometric analyses, microscopic observations of cross-sections of stems, measurements of chlorophyll fluorescence and gas exchange, and determination of ^{13}C isotope discrimination in various plant organs.

The preliminary results suggest that limiting light access to the stem inhibits plant growth and development, which may indicate the significant role of chloroplast-containing cells in stems in regulating these processes.

The results obtained allow for an evaluation of the current understanding of the role of green tissues in non-woody plant stems and their underestimated influence on the plant's life cycle.

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O-113

Root-based inorganic carbon uptake boosts photosynthetic activity and osmotic stress tolerance

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Keywords: carbon metabolism, photosynthesis, drought

Root-based inorganic carbon uptake could boost photosynthetic activity by improving its carbon supply and increase drought tolerance by providing carbon dioxide when stomata are closed. During our work, physiology and mechanism of root-based inorganic carbon uptake of barley (*Hordeum vulgare* 'Golden Promise') and *Arabidopsis thaliana* (Col) have been characterised. Growth-promoting hydroponics-based inorganic carbon treatment has been optimised, it then served as basis for further experiments. The bicarbonate treatment also alleviated the effects of osmotic stress caused by PEG, though only in Arabidopsis. Growth promotion was achieved by supplying 2 mM $NaHCO_3$ at low pH (5.6). It increased shoot fresh weight of barley by 20%, but shoot fresh weight of Arabidopsis by 50%. Using the optimal treatment, we proved by heavy carbon labelling and fluxomic analysis that the plants took up and fixed the supplied carbon. The level of ^{13}C-labelled aspartic acid (indirect product of PEPC-based fixation) slightly increased in root/xylem sap samples in both species but the level of labelled sucrose (main photoassimilate, indirect product of Calvin-cycle-based fixation) greatly increased only in the phloem sap in Arabidopsis. Nevertheless, the level of many amino acids (aspartic acid, threonine, serine, cysteine) increased in the barley shoot indicating transport of the root-produced aspartic acid and possible release and refixation of the supplied carbon. Transcriptome analysis was also applied to understand the background of the growth promotion and to find possible bicarbonate transporters. According to the transcriptome sequencing data, the observed enhancement in the growth of Arabidopsis may be orchestrated by trehalose-6-phosphate signalling and supported by augmented nitrogen and sulfur assimilation. In case of barley, response to auxin, tricarboxylic acid metabolic process and gluconeogenesis as activated pathways are worth to be mentioned. Nitrate, phosphate and sulphate transporter genes were upregulated in the two species as result of the bicarbonate treatment and were partially validated for bicarbonate transport. The growth, osmotic stress tolerance and fluxomic results argue for that the barley variety studied here, Golden Promise might not have the same effective root-based bicarbonate uptake mechanism like Arabidopsis. Future over-expression of bicarbonate transporters in barley could boost its drought tolerance.

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O-114

Key factors enhancing growth by integrating green and far-red light in LED lamps

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Keywords: indoor vertical farming, morphological and photosynthetic acclimation, green and far-red light

Indoor vertical farming relies on precise environmental control to optimize plant growth, with light being a key factor. The adoption of LED technology has improved energy efficiency, allowing precise control over light spectra and intensity. Traditionally, controlled environments primarily supplied red and blue (RB) wavelengths due to their high absorption by

photosynthetic pigments, while green (G) and far-red (Fr) light were excluded to minimize energy costs. However, G and Fr light enhance plant growth by penetrating the canopy, stimulating photosynthesis of shaded leaves and triggering morphological responses through photoreceptor activity. Moreover, maximizing photosynthesis alone does not necessarily lead to greater biomass accumulation, raising the unresolved question of which key factors drive plant growth and how they interact.

In this study, we explored how G and Fr light shape morphometric responses, photosynthetic and respiratory acclimation in *Lactuca sativa*. Building on previous research defining optimal RB ratios for vertical farming, we tested different intensities and proportions of G and Fr light under both discontinuous and continuous emission spectra. To evaluate photosynthetic performance, we conducted fluorimetry and gas exchange measurements, while biochemical assays provided insights into photosynthetic apparatus composition. Additionally, we analyzed morphometric traits related to relative growth rate (RGR) to assess the broader impact of light quality on plant growth. After just two weeks of acclimation, light spectra incorporating G and Fr wavelengths boosted biomass accumulation by over 30% compared to plants grown under RB light. RB light induced a high-light-like response, increasing net CO_2 assimilation and electron transport rate, whereas G and Fr light led to low-light-like acclimation, following an opposite trend. Morphological acclimation to RB light resulted in thicker leaves and a lower shoot/root ratio, increasing the carbon cost of leaf expansion. In contrast, G and Fr light induced the opposite trend, ultimately enhancing RGR.

Our results highlight the need to integrate photosynthetic, morphological, and respiratory acclimation to optimize lighting strategies for indoor farming. Incorporating G and Fr wavelengths in LED systems enhances plant growth and resource efficiency to better meet modern agricultural demands.

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O-115

Sugar infusion in trees: Possibility, effects and applications

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Keywords: external sugar addition, leaf gas exchange, leaf senescence, regulatory mechanism, source-sink

Stress (e.g. drought)-induced carbon (C) limitation remains one of the possible physiological mechanisms leading to tree mortality and forest dieback (McDowell *et al.*, 2022). The exogenous sugar addition may increase C availability in C-limited trees, and may also provide new opportunities to investigate tree C balance, source-sink dynamics, and the role of non-structural carbohydrates (NSCs) in stress responses. In a series of experiments, we infused ^{13}C-labeled glucose into the xylem of C-limited bonsai (*Ficus macrocarpa* L. f.), sycamore maple (*Acer pseudoplatanus* L.), and Scots pine (*Pinus sylvestris* L.) to assess the uptake, transport, and metabolic fate of the added sugars (Zhang *et al.*, 2023). We traced the ^{13}C label in plant organic matter, respiration, and structural growth (cellulose), and measured NSC concentrations and photosynthetic responses. Our results demonstrate that bonsai, maple, and pine trees can take up, transport, and metabolize exogenous sugars infused, with significant incorporation into biomass in maple and pine. However, sugar addition triggered photosynthetic downregulation and leaf senescence in maple (Zhang *et al.*, under review), but not in pine, highlighting species-specific differences in C uptake and allocation. Moreover, while sugar infusion increased leaf NSC concentrations, it also exacerbated root C limitation in maple when photosynthesis was impaired. Conversely, pine maintained stable photosynthetic rates and needle NSC concentrations in response to sugar addition, showing the potential of sugar infusion to mitigate root C limitation in C-limited pines (Zhang *et al.*, in revision). These results suggest that exogenous sugar supply may mitigate root C limitation only if photosynthetic performance remains stable. Our studies suggest exogenous sugar addition as a research tool for tree C relations and a practical strategy to alleviate stress-induced C limitation. Controlled supplementation may enhance tree survival in conservation, especially for historic and landmark trees, as well as monumental trees

in urban areas, parks, and avenues that are vulnerable to defoliation, drought, or other environmental disturbances.

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O-116

Regulation of photosynthesis in far-red insensitive mutants of tomatoes

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Keywords: energy-partitioning, far-red, phytochrome, photoprotection

Far-red (FR) light plays a significant role in influencing leaf photosynthesis and photoprotection under intense light exposure, yet the precise mechanisms through which FR signaling regulates photosynthetic processes remain unclear. To explore the function of phytochrome A (PHYA) in modulating plant responses to high light conditions, we subjected both wild-type (WT) tomato plants and phyA mutants (*phya*) to high-intensity white light (1000 µmol m^{-2} s^{-1}), with or without additional FR light (250 µmol m^{-2} s^{-1}). We examined how FR light affects electron transport and the Calvin-Benson cycle in these plants. Our findings revealed that in the absence of PHYA, exposure to FR light led to an increase in the quantum yield of non-photochemical quenching (Φ_{NPQ}) while reducing the quantum yield of non-regulated energy dissipation (Φ_{NO}). This shift in energy allocation helped mitigate the decline in the quantum yield of photosystem II (Φ_{PSII}). Additionally, FR light exposure in *phya* mutants was associated with greater energy dissipation through NPQ, an increase in donor-side limitation (Φ_{ND}), reduced effective quantum yield of photosystem I (Φ_{PSI}), and enhanced oxidation of P700. FR light also influenced the Calvin-Benson cycle, with phya mutants exhibiting a more pronounced increase in the maximum carboxylation rate of Rubisco (VCmax) compared to WT plants. However, unlike WT, the *phya* mutants did not show FR-induced increases in the maximum electron transport rate (J_{max}) or triose phosphate utilization (TPU). Overall, our study highlights the differential response of tomato plants to high light exposure depending on PHYA presence, underscoring the essential role of FR signaling in regulating plant adaptation to intense light conditions.

O-117

Salicylate profiling in plants in response to bacterial infection

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Keywords: salicylates, liquid chromatography, tandem mass spectrometry

Salicylic acid (SA) is a plant hormone regulating defences against pathogens, stress responses, and growth processes. The involvement of SA biosynthetic pathways and the extent of its metabolism preceding and following SA accumulation are still being investigated across various plant species.

Here we present a novel, optimized liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantitative analysis of salicylates, including SA, its biosynthetic precursors, and metabolites. The chromatographic method was optimized to separate the target analytes from their isomers and interfering matrix components while ensuring an acceptable peak shape and enhancing MS detection sensitivity. A sample preparation step was developed to extract and selectively purify the analytes, minimize matrix interference, and pre-concentrate the analytes to improve MS detection. We first applied the method to describe the dynamics of biosynthesis and conversion of

salicylates in *A. thaliana* leaves infected by bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000.

Measuring salicylate concentrations provides valuable insights into plant physiological processes, helping to better understand plant responses to environmental changes, diseases, and pests. This knowledge can contribute to the development of strategies for enhancing plant resilience and stress tolerance as well as improving crop protection and breeding practices.

This work was supported by the Czech Science Foundation (Grant No. 22-17435S).

O-118

3-in-1 *in vivo* reporter system: simultaneous detection of Auxin, GA and ABA phytohormonal responses and crosstalk in rice

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Keywords: ABA, Auxin, GA, luciferase, GUS, phytohormone, promoter, rice

Analytical determination provides accurate quantification of phytohormone contents; however, the extraction procedure is time-consuming, and the antagonism, dynamic changes, and crosstalk among phytohormones may not be detected. Therefore, a simple system for the simultaneous detection of phytohormone responses during seed germination or environmental stress is highly needed. To address this, we have developed a simple, sensitive, and reliable in vivo Auxin, GA, and ABA reporter system (AGA system) in this study. The AGA system consists of an auxin-responsive GUS reporter and a dual-luciferase reporter system responsive to GA and ABA. Transgenic rice lines were generated, harboring a GUS reporter driven by the auxin-responsive *DR5* promoter, a firefly luciferase reporter under the control of a synthetic promoter containing two copies of the *ABRC*, and a Renilla luciferase reporter driven by the GA-responsive *Amy32b* promoter. This system is valuable for studying the effects of exogenous factors (e.g., abiotic stresses, xenobiotics, root exudates, and elicitors) and endogenous factors (e.g., developmental stages and tissues) on dynamic changes in auxin, GA, and ABA responses and hormonal crosstalk. The potential applications of the AGA system will be discussed in the presentation.

This work was supported by the National Science Technology Council of Taiwan: MOST 108-2313-B-002-055-MY3 and MOST 111-2313-B-002 -031 -MY3

O-119

A comparative analysis of receptor like kinases in chlorophyta reveals the presence of putative cell wall integrity sensors

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Keywords: microalgae signal transduction, receptor functional divergence, algae-plants evolutionary conservation

Cell walls (CWs) are extracellular structures that maintain cell integrity and sense environmental signals, playing crucial roles in development, defence, and adaptation. The Cell Wall Integrity (CWI) monitoring system comprises mechanisms that enable cells to perceive environmental stimuli and adapt by adjusting their structures and compositions. In plants, Receptor-Like Kinases (RLKs) are well-characterized sensors of CWI, whereas their counterparts in microalgae remain largely unidentified. This knowledge gap is primarily due to limitations in analytical methods.

In my presentation, I will show how we have used advanced bioinformatics tools and AI-driven algorithms to map the distribution of RLKs across the *Chlorophyta* phylum (microalgae), characterise their structural and functional properties, and investigate their relationship with known plant counterparts. Our analysis revealed that RLKs are widespread among *Chlorophyta*. Using structural and remote homology analyses, we identified multiple domains associated with CWI perception in plants. Interestingly, the classification of these domains highlighted the presence of diverse functional groups, with role in: (i) protein-protein interaction (e.g., ARM, LRR), (ii) CW remodelling (e.g., glycosyl hydrolases, lyases and carbohydrate-binding) as well as (iii) CW-related domains putatively involved in mechanosensing (e.g., LPXTG, Fibronectin).

This work represents a first step towards understanding the evolution of RLKs from microalgae to vascular plants and provides a novel framework for investigating their functional diversification. I will also discuss how this knowledge could support targeted modifications of CW porosity and flexibility, with potential applications in stress adaptation and biomass use.

This work was supported by JCSMK23-0228 grant from Kempestiftelserna, Sweden, and by grants from the Knut and Alice Wallenberg Foundation and Vetenskapsrådet, Sweden.

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O-120

The light in the night: Role of reactive oxygen species and defence-related phytohormones in the action of nocturnal red light

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Keywords: ethylene, jasmonic acid, superoxide

Plant infections caused by fungi such as *Botrytis cinerea* result in significant crop losses worldwide each year. Growers most commonly use chemical and fungicide-based methods to control grey mould both in greenhouses and in the field, but alternative and combined methods of plant disease management have become a key area of plant biological research today. This study aims to better understand the red light regulated plant defence mechanisms of tomato plants, in particular the effects of red light at night when most phytopathogens are highly infectious. Our results showed that superoxide production increased significantly immediately after red light exposure and, together with hydrogen peroxide levels, was highest at dawn after 30 min of nocturnal red light treatment. In parallel, red light induced the expression and increased the activities of several antioxidant enzymes. The role of reactive oxygen species was further investigated in phytochrome mutants. Nocturnal red light did not affect salicylic acid, but increased ethylene and jasmonic acid levels immediately after illumination, whereas abscisic acid levels increased 3 h after nocturnal red light exposure at dawn. The role of ethylene was further investigated in the ethylene receptor mutant *Never ripe* leaves. Based on the RNAseq data, red light immediately increased the transcription of several chloroplastic *chlorophyll a-b binding protein* and circadian rhythm-related genes, such as *Constans 1*, *CONSTANS interacting protein 1* and *zinc finger protein CONSTANS-LIKE 10.* In addition, the levels of several transcription factors were also increased after red light exposure, such as the *DOF zinc finger protein* and a *MYB transcription factor* involved in the regulation of circadian rhythms and defence responses in tomato. In addition to the identification of these key transcription factors in tomato, the application of red light at night for one week not only reactivated key antioxidant enzymes at the gene and enzyme activity level at dawn, but also contributed to a more efficient and successful defence against *Botrytis cinerea* infection in an ethylene-dependent manner. At the same time, the long-term effects of night-time red light application on the metabolism of young and old leaves of the plants, based on metabolome analysis, were significant and different.

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O-121

Redox- and light spectrum-dependent modulation of microRNAs and their targets during stress adaptation

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Keywords: ascorbate, blue light, far-red light, glutathione, salicylate

Changes in the environmental conditions modify the redox environment of plant tissues, which alteration influences the expression of miRNAs. Their transcriptionally and post-transcriptionally regulated target genes in turn modify the metabolism, growth and development. The aim of our study was the identification of redox-responsive microRNAs and their target genes, and the determination of light spectrum-dependent regulatory mechanisms of stress adaptation. For the

selection of such microRNAs, the redox environment of wheat shoot tissues was shifted into a more oxidizing state by H_2O_2 based on the redox state of glutathione (GSH) pool. This resulted in a minimum 1.5-fold change in the expression of 70 miRNAs influencing 86 target genes based on degradome sequencing. In addition, 6722 possible target genes were determined with bioinformatics tools, and the H_2O_2-responsiveness was confirmed for 1647 genes by transcriptome analysis. They regulate redox processes, transcription, protein phosphorylation and degradation. Among them, the miR3106a-responsive beta-carotene isomerase is involved in strigolactone biosynthesis. Further redox-controlled miRNAs were identified by the comparison of the miRNA profile in wild-type Arabidopsis plants and lines with decreased ascorbate (Asc), GSH or salicylate (Sal) level. GSH deficiency did not affect the miRNA levels, but lack of Asc and Sal decreased the amount of 9 and 44 miRNAs, respectively. Four miRNAs were in turn upregulated by Asc or Sal deficiency. Their predicted target genes participate in the metabolism of nucleic acids, aromatic compounds, nitrogen and sulphate. A greater activation of the Asc- and Sal-responsive miR395 (controlling GSH synthesis) was observed after sulphur starvation in far-red light compared to white and blue light in wild-type and GSH-deficient Arabidopsis lines. Among its target genes, sulphur starvation decreased the expression of *ATP sulfurylase 4* in far-red light. Based on these observations, the Asc-GSH cycle may mediate the effect of spectral changes on miR395. Using bioinformatics modelling, a regulatory network of redox-responsive miRNAs and their targets was created, which indicates the complexity of the mechanism of adaptation to changing environmental conditions by adjustment of metabolism. The continuation of the research may be the clarification of the operating mechanism of some miRNA – transcription factor regulatory modules within this network. Their chemical or genetic modification can be used for the reduction of stress-induced loss of the yield quality and quantity in the future.

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O-122

Light and temperature-dependent shoot-root signalling pathways in cereals

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Keywords: acclimation, cereals, cold, light, shoot-root signalling

Acclimation to low temperatures is one of the most important stress responses in plants. Light also determines the effectiveness of acclimation to cold. When cold hardening takes place at low light intensities, both the frost resistance of winter cereals and the chilling tolerance of cold sensitive plants are lower. The main aim of the presented work was to better understand, how the low temperature signal from the leaves may affect the stress responses in the roots, and how the light conditions modify certain stress acclimation processes cereals. To achieve the goals, in the 1st experiment rice plants grown at 27°C were exposed to low temperatures (12 °C) with different light intensities, so that in the case of some plants only the leaves received the cold, while the roots remained at the control temperature. Using the RNAseq technique from the roots of plants grown under normal growth light conditions, several differentially expressed genes were found in different comparisons. Exposure to low temperature led to more down-regulated than up-regulated genes. Comparison of the roots of the leaf-stressed plants with either the whole cold-treated or the control plants revealed that nitrogen metabolism and nitric oxide-related signalling, as well as the phenylpropanoid-related processes were specifically affected in them. Real-Time PCR results focusing on the COLD1 and polyamine oxidase genes, metabolomics targeting hormonal changes and phenolic compounds showed that not only cold exposure of the leaves, either alone or together with the roots, but also the light conditions may affect certain stress responses in the roots of rice plants both at gene expression and metabolite levels (Gholizadeh *et al.* Physiol Plant. 2024, 176(5):e14541. doi: 10.1111/ppl.14541.).

In the next experiment, wheat plants were grown in a growth chamber at 22/20 °C day/night temperatures with 16h daily illumination provided by LED system. Part of the plants were grown under white light (W) light, while other plants were exposed to elevated blue (B) light illumination with the use of the same total light intensity.

The roots were kept dark during the whole experiment. After 13 days, plants were exposed to 5 °C for 1 week. Parallel with this, while half of the plants stayed under the same light conditions during the cold treatment at 5 °C as they were previously grown, others were shifted from W to B and vice versa, from B to W. Measurements of the chlorophyll-a fluorescence parameters, targeted and untargeted HPLC analyses performed on the leaves and roots of plants, completed with gene expression analyses demonstrate that blue light may induce specific cold acclimation processes in wheat leaves, and the effects of light signal can also be detected in roots.

This work was supported by a grant from the Hungarian National Research, Development, and Innovation Office (K 142899).

O-123

Improving the resilience of chestnut plants to climate change: The role of stress priming

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Keywords: Castanea sativa; abiotic stress; mitigation strategies; drought

The chestnut tree, a key forest crop in Portugal, is increasingly threatened by emerging diseases and climate change. Given the rising frequency of heat waves and drought episodes in the Mediterranean region, developing sustainable and effective strategies to enhance chestnut trees' resilience to climate change is crucial. Within this context, stress priming has been identified as a potentially effective approach, as plants pre-exposed to mild stress conditions often exhibit increased resilience to future adverse conditions. However, while this concept has been widely studied in herbaceous species, it remains largely unexplored in trees. Thus, the main goal of this study was to evaluate the effectiveness of drought-induced stress priming in enhancing chestnut tolerance to water stress. To achieve this, young chestnut plants were subjected to either a priming treatment (three weeks under mild drought conditions at 35% field capacity, followed by a two-week recovery period under control conditions) or left non-primed. Subsequently, both primed and non-primed plants were exposed, or not, to drought stress (four weeks at 25% field capacity). The results showed that neither stress exposure nor priming significantly affected the biometric parameters of plants. However, the drought-induced reduction in leaf area was significantly mitigated in primed plants. Additionally, compared to their non-primed counterparts, primed plants under both control and stress conditions exhibited higher photochemical efficiency and lower non-photochemical quenching while also showing smaller declines in transpiration, stomatal conductance, and carbon assimilation, indicating an overall improvement in photosynthetic performance. The assessment of redox status further supported these findings, as drought stress led to increased reactive oxygen species levels and lipid peroxidation in non-primed plants, whereas primed plants showed no signals of oxidative damage. Furthermore, while non-primed plants exposed to drought increased their levels of antioxidant metabolites, such as proline and glutathione, primed plants did not alter the content of these compounds when facing stress. Overall, this pioneering study demonstrates, for the first time, that drought-induced priming enhances chestnut resilience to water stress, highlighting stress priming as a promising strategy to improve chestnut tree adaptation to climate change. Currently, a new trial is underway to unravel the underlying mechanisms, particularly proteomics features, responsible for this priming-induced tolerance.

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O-124

Unraveling sulphur metabolism and miR395 dynamics in salt- tolerant and sensitive rice varieties

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Keywords: salt stress, rice, sulphur metabolism, miRNA

Increasing soil salinization poses a major challenge to global agriculture. Rice (*Oryza sativa*), a staple crop feeding over 50% of the world's population, is one of the most salt-sensitive cereal, with salinity stress severely affecting its growth, development, and grain yield. A key component of plant stress adaptation is the regulation of reactive oxygen species (ROS). Comparative analysis of salt stress responses in tolerant and sensitive rice varieties highlights glutathione (GSH) as the primary redox metabolite involved in salt stress response. Since GSH is the major organic sulphur compound in plants, this study focused on sulphur metabolism and its transcriptional and post-transcriptional regulation in four Italian rice varieties that differ in salinity tolerance. Our findings indicate that salt stress causes alteration of sulphur translocation and assimilation mainly in sensitive varieties. The differential gene expression of key enzymes belonging to sulphur metabolism was investigated by analyzing the methylation profile of their corresponding genes and assessing the role of a previously identified microRNA (miR395) as a key post-transcriptional regulator of genes involved in sulphate translocation and assimilation (sulphate transporter *SULTR2;1* and ATP sulphurylase *ATPS*). MiR395 exhibits a more rapid and effective post-transcriptional regulation in tolerant varieties, ensuring efficient sulphur assimilation and GSH production. The obtained data suggest a relationship between sulphur metabolism and salt stress tolerance, highlighting the need for further investigation to enhance plant resilience and ensure stable rice production.

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O-125

All in one: A serine carboxypeptidase-like protein catalyzes synthesis of chicoric and isochlorogenic acids in *Cichorium intybus*

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Keywords: specialized metabolism, caffeic acid esters, chicory

Chicory (*Cichorium intybus L.*), a member of the *Asteraceae* family, has a long history of use in traditional medicine. Its health benefits may be largely due to its unique composition of caffeic acid esters, mostly chlorogenic (CQA), isochlorogenic (DiCQA), caftaric (CTA) and chicoric acid (DiCTA). Caffeoylquinic acids (CQA and DiCQA) are commonly found in plants, where they play roles in defense against pathogens and as efficient scavenger of reactive oxygen species upon UV exposure. On the other hand, caffeoyltartaric acids (CTA and DiCTA) are far less common but found in large amounts in chicory leaves. All these compounds have multiple hydroxyl functional groups which may explain their various biological activities such as antioxidant, anticancer, antioxidant and anticancer properties, making chicory a valuable source of bioactive molecules.

Using a candidate gene approach, we identified an enzyme from the serine carboxypeptidase-like (SCPL) family, named CiCAS. This vacuolar protein catalyzes the transfer of a caffeoyl group from CQA to either CTA or another molecule of CQA, to form diCTA or diCQA, respectively. These results were obtained *in vitro* using purified recombinant protein and further validated *in vivo* in tobacco and yeast. In addition, qRT-PCR analyses showed that *CiCAS* expression pattern matches with the accumulation of chicoric acid and generated CRISPR-Cas9 knocked out (KO) lines display drastic reduction in chicoric acid content confirming the physiological role of this enzyme. Additional experiments are under progress to identify new genes involved earlier in this pathway and explore evolutionary mechanisms in SCPL proteins that led to this unique dual activity. In parallel, we are

developing a yeast-based bioproduction system to synthetize high levels of these molecules.

These results further confirm the catalytical diversity of acyltransferases in plants that may behave as key drivers for the formation of complex molecules with interesting biological activities. In the future, generated chicoric acid deficient lines will serve as a model to study the role of this compound in plant responses to environmental stresses. This may help breeders to select plants with higher nutritional properties and improved fitness in agricultural systems. Meanwhile, microbial bioproduction platforms may provide a sustainable source of these high value compounds for human health research and potential therapeutic applications.

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O-126

Elucidation of the biosynthetic pathway of two C-glucosyl flavones accumulated in winter flax (*Linum usitatissimum* L.)

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Keywords: flax, cold stress, C-glycosyl flavones, biosynthesis

Linum usitatissimum (flax) is a plant cultivated for its fibers or for its seeds rich in essential fatty acid omega-3. Lately, fiber yields in France have been drastically reduced due to water deficit during the first stage of spring flax development. As a consequence of climate change, these accidents may occur more and more frequently. In this context, the selection of more winter varieties, less exposed to drought period during their growth, is required but impaired by the lack of information regarding flax cold tolerance. Recently, two *C*-glucosylated flavones, swertisin and swertiajaponin, were found to be specifically accumulated in aerial parts of winter varieties and, as such, considered as putative cold tolerance biomarkers. Recently, we succeed to identify the molecular determinants responsible for their synthesis. Through a quantitative trait loci (QTL) analysis carried out from the 148 recombinant inbred lines resulting from the cross between a winter and spring flax variety, a type I OMT candidate (LusOMT1) was identified and characterized. *In vitro*, recombinant proteins methylate specifically the *C*-glycosyl flavones isovitexin and isoorientin at the 7-hydroxyl position to form swertisin and swertiajaponin. Additionally, transient overexpression of LusOMT1 in *Nicotiana benthamiana* that does not naturally accumulate these molecules, resulted in the production of swertisin and swertiajaponin when the substrates isovitexin and isoorientin were co-infiltrated. To definitely validate the biochemical role of this enzyme in flax, experiments are now under progress to invalidate their synthesis in winter varieties or to introduce their synthesis in spring varieties. Furthermore, new candidate genes were identified to complete the whole biosynthethic pathway of swertisin and swertiajaponin. These data should pave the way to the improvement of winter flax selection by breeders and help reduce the impact of climate change on flax fiber production in the future.

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O-127

Effect of a pre-harvest treatment with harvistatm on the nutraceutical quality and volatile compound profile of apples

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Keywords: 1-methylcyclopropene (1-MCP), shelf-life, nutritional value

The formulation of a pre-harvest treatment including the active ingredient 1-methylcyclopropene (1-MCP), Harvista™ (Harvista) effectively delays fruit ripening. This

study aimed to investigate the effects of Harvista on the ripening process, nutraceutical properties, and aromatic composition of three apple varieties ('Royal Gala', 'Golden Delicious', and 'Rosy Glow') at harvest, after 3 months of cold storage (0.5 ºC), and during a subsequent shelf-life of 7 d after cold storage (20 ºC). Three experimental groups were established: (i) control fruit (CT), (ii) fruit treated with Harvista and harvested on the same day as the CT, (iii) fruit treated and then harvested at a starch index (SI) of 7-8. Quality parameters, ethylene production, nutraceutical profile, and volatile compounds were analysed at different sampling points.

The chlorophyll absorbance index (IAD), SI, firmness, total soluble solids (TSS), and Hue indicated that Harvista was effective in delaying fruit ripening on the tree, thereby enhancing its storage potential. These characteristics at harvest were well correlated with ethylene production. After cold storage and subsequent shelf life, the treated fruit exhibited a reduced loss of firmness and better colour retention. The most significant effects were observed in 'Rosy Glow' apples, in which the treated groups remained less ripe throughout the study. Globally analysing all of the points for three varieties, the treatment did not negatively affect the concentration of water-soluble vitamins, antioxidant capacity, vitamin C, or phenolic compounds. Similarly, in general, the volatile compounds were not adversely altered, so the treated fruit presented a similar profile to that of the CT fruit.

Pre-harvest application of Harvista appears to offer an effective treatment for extending the harvest window and improving fruit preservation without compromising its nutraceutical properties.

O-128

Genetics and biochemistry of sticky trichomes

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Keywords: Trichomes, sticky, sphingolipids

Most plant species have trichomes or epidermal hairs, which can have diverse structures and functions in providing resistance to pests, UV radiation, or drought. The genus *Antirrhinum* (snapdragon) provides a useful research system in which to identify and study the genetics and biochemistry of trichomes because its species differ in trichome morphology and the presence or absence of a secretory gland in the trichomes.

Antirrhinum hispanicum trichomes release a viscous secretion which exhibits a remarkable stickiness and is able to trap insects. The sticky secretion is insoluble in water and does not dry or harden at ambient temperature. Liquid chromatography coupled to mass spectrometry (LC-MS) analysis identified a mixture of compounds belonging to the same family: Sphingolipids

Comparative transcriptome analysis of sticky *A. hispanicum* and non-sticky *A. majus* epidermis supported this by revealing that expression of genes involved in fatty acid metabolism was enriched in *A. hispanicum*.

To identify the genetic basis of stickiness and its evolution, *A. hispanicum* was crossed with non-sticky A. majus. F1 hybrids and three-quarters of F2 plants were not sticky, indicating that stickiness is determined by the recessive allele of a single gene. The sticky gene has been mapped to a short region of the genome by backcrossing the F1 to *A. hispanicum* and sequencing DNA from pools of sticky and non-sticky plants. The sticky will be identified by a combination of expression analysis and virus-induced gene silencing of candidates in the region.

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O-129

Polyamines in the "spotlight"

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Keywords: daily light period, light quality, light spectrum, polyamine metabolism, stress

Polyamines (PAs) are aliphatic amines found in all living cells. They occur in much higher quantities than plant hormones, and their levels show significant differences depending on the plant species, organ and developmental stage. Previously, PAs were attributed a simple protective effect, since due to their cationic nature, they can interact reversibly with negatively charged macromolecules, thereby stabilising their structure, especially under stress conditions. However, PAs as signalling molecules are involved in the regulation of various basic cellular processes, such as cell division, differentiation, proliferation, cell death, DNA and protein synthesis, and gene expression. Most of the previous studies focused on the beneficial effects of PA treatments and emphasised the correlation between stress tolerance

and increased PA content. Although an immediate increase in PA levels is a common stress response, it is also possible that their increased amount triggers a stress effect, so the statement that the more the better cannot be generalised. The metabolism of PAs is extremely dynamic due to the PA-cycle, moreover, their metabolism is related to or interacts with several other protective compounds.

The relationship between PAs and photosynthesis has been demonstrated at several levels. They can influence pigment synthesis, gas exchange and net photosynthesis, thus have positive effects even under stressless conditions. Nevertheless, it was demonstrated that different characteristics of light (light period, light intensity or spectral composition) influenced PA metabolism. Recently we also found that the roborative effect of the PA treatments depends on light growth conditions. In addition, the outcome of the PA treatments under stress conditions, for example, cadmium stress, is influenced by light spectral composition, which could be the result of a metabolic shift between PAs and other compounds. However, PA treatment can induce changes not only at the metabolite level but also in the DNA methylation pattern. Our results showed that under white light conditions, the putrescine treatment induced more alteration, while blue light had higher effects on methylation than the putrescine treatment. We also demonstrated that although the PhyA protein is considered primarily as a far-red sensor, the responses of wild-type and *phyA* mutant *Arabidopsis* plants are partly different and depend on the light spectral conditions, namely under blue light conditions during spermine excess PhyA inhibit the back conversion and subsequently downregulate putrescine synthesis.

The beneficial effects of PA treatments depend on the initial level of the endogenous, individual PAs and the intensity of the PA metabolism, which are environment-, development, species-, and even genotype-dependent. The proper shift in PA levels required a well-maintained dynamic balance of PAs through fine-tuning of PA metabolism (synthesis and catabolism, uptake and conjugation of PAs).

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O-130

Polyamine metabolism in microgreen plants during salt stress

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Keywords: polyamines, microgreens, salt stress

Polyamines are essential plant growth regulators in all living organisms. Their role in plants is crucial and evidenced during growth and development and in stress conditions. Microgreens are young seedlings with rapid growth and valuable secondary metabolites. Despite the controlled production of microgreens influencing their bioactive compounds in vertical farming systems have great interest nowadays, the polyamine metabolism of these fast growing plants still requires investigation. Our approach was to study the polyamine metabolism in microgreen plant species using different growth media and salt stress treatments. We have found some evidences for plant species-dependent alterations in polyamine homeostasis including the hypusination, a spermidine dependent metabolic posttranslational modification of eukaryotic translation factor 5A (eIF5A) (Pálfi et al., 2021). Hypusine, rare amino acid can activate eIF5A uniquely, is synthesized by two enzymatic reactions, catalyzed by deoxyhypusine synthase and deoxyhydroxyhypusine hydroxylase. Our research group provided evidence about the salt stress induced alteration of polyamine catabolism and hypusination by using the pharmacological inhibitor of deoxyhypusine synthase, GC7 in *Arabidopsis thaliana* (Szepesi et al., 2023). Our results from the current study enhance our knowledge about the stress-dependent regulation of hypusination by investigating the steps of hypusine synthesis in microgreens during salt stress.

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O-131

Cadmium stress in *Salvia* species: Unveiling the phenolic profile, antioxidant potential and toxicity assessment

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Keywords: cadmium toxicity, antioxidant activity, polyphenols, medicinal plants, heavy metal stress

The accumulation of heavy metals in agricultural soils poses an environmental challenge, reducing crop yields and compromising food safety. High metal concentrations inhibit plant growth by affecting key physiological processes, including secondary metabolism. However, medicinal and aromatic plants have been proposed as alternative crops for contaminated soils, where heavy metals may enhance secondary metabolite synthesis.

This study examines cadmium contamination's impact on *Salvia officinalis* L. and *Salvia ceratophylloides* L., focusing on polyphenol content, antioxidant properties, and extract toxicity. While *S. officinalis* is well known, *S. ceratophylloides*, endemic to Southern Italy, remains largely unexplored. Two cadmium concentrations (5 mg/kg and 10 mg/kg) were tested, reflecting the Italian regulatory limit (100%) and twice that value (200%). Growth, gas exchange, chlorophyll fluorescence, polyphenols (via LC–DAD/ESI–MS), antioxidant activity (DPPH, Fe^{2+} chelation assays), and toxicity (Artemia salina lethality bioassay) were assessed.

Results indicate cadmium reduces total polyphenol content, except for caffeic acid, which increases under stress. *S. ceratophylloides* extracts exhibited stronger radical scavenging and metal chelation than *S. officinalis*, even under non-stressed conditions. Despite greater sensitivity to Cd stress, *S. ceratophylloides* showed higher antioxidant activity. The *A. salina* assay confirmed no toxicity in *S. ceratophylloides* at 1000 µg/mL (100% survival), while *S. officinalis* extracts exhibited moderate to high toxicity.

These findings highlight *S. ceratophylloides* as an antioxidant-rich species with no detectable toxicity, making it a promising candidate for environmental remediation and bioactive compound development.

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O-132

Biotechnological production of a yellow carrot cell culture enriched in phytosterols and phenolic compounds and evaluation of its potential biostimulant effect in tomato seeds

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Keywords: biotechnological production, higher plant-derived biostimulants, yellow carrot cell cultures

Climate change and the expanding global population are putting pressure on modern agriculture to sustain production on increasingly limited arable land. Plant biostimulants are emerging as a safe and environmentally friendly solution to enhance crop production and yield under challenging conditions. Among these, higher plant-derived biostimulants (hPDBs) are particularly notable due to their advantages in sustainability, production costs, safety, and, most importantly, the wide range of bioactive compounds they contain [1]. In this work, yellow carrot cell suspensions were used to increase their bioactive compound content through biotechnological production. Using elicitation techniques [2], the effect of different types of beta-cyclodextrins (β-CD) on phytosterol production was evaluated. The highest levels of phytosterols were obtained when yellow carrot cell suspension were incubated in the presence of 25 mM of methylated-β-CD (Me-β-CD) for 7 days. To increase the total phenolic content (TPC), methyljasmonate (MJ) addition was evaluated, alone or in combination with 25 mM Me-β-CD. The results demonstrated that the combination of 25 mM Me-β-CD and 100 µM MJ (CDMJ) increased phytosterol production and TPC in yellow carrot cell suspensions. Then, we evaluated the potencial of a novel type of hPDB

based on a yellow carrot cell culture enriched in phytosterols and phenolic compounds with ability to alleviate salinity stress in tomato seeds.

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PLANT BIOLOGY EUROPE 2025

BUDAPEST

POSTER PRESENTATIONS

APPLICATION OF GENETIC IMPROVEMENTS

P-1

Transgene-free gene specific editing of maize genome with synthetic oligonucleotides

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Keywords: maize, Oligonucleotide Directed Mutagenesis, chemical modification, protoplast, PEG, cationic polymer

Oligonucleotide-directed mutagenesis (ODM) is a simple gene editing method that uses short, single-stranded oligonucleotides to introduce precise mutations into the target genes, but so far with low efficiency. Improving efficiency of ODM is of fundamental importance for wide application of this gene-editing approach in plant science and breeding.

We intend to increase the efficiency of ODM by several approaches; chemical modification of oligonucleotides to increase their *in vivo* stability, delivery of oligonucleotides into maize protoplasts using PEG and cationic polymers and comparison with biolistic method, by relaxing the chromatin structure and use of HDR enhancers. The effect of different conditions on the efficiency of gene editing was monitored by the correction of the STOP codon in the mutant GFP (*mGFP*) gene, which resulted in GFP positive cells that could be easily detected by fluorescence microscopy in few days. *mGFP* gene correcting oligonucleotides were co-delivered with oligonucleotides designed to introduce STOP codons into our target genes GA20-oxidase (*GA20ox*) or hexokinase1 (*HXK1*), which may lead to enhanced drought tolerance in maize. SZ17 maize cell line capable of plant regeneration was used in these experiments. Introduction of the targeted mutation into *mGFP*, *GA20ox* and *HXK1* genes was proven by NGS sequencing method.

This work was supported by the National Research, Development and Innovation Office of the Hungarian Government through the RRF-2.3.1-21-2022-00007 grant for the "National Laboratory Program of Agro-Biotechnology and Precision Plant Breeding to Support Food Safety".

P-2

Tiny hairs, big impact: Molecular insights into tomato trichome density

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Keywords: trichome, ViGS

Most flowering plants possess trichomes, or hair-like structures, on their surfaces, which play a critical role in protecting against pests and producing valuable chemicals such as pharmaceuticals and flavours. This has increased interest in enhancing trichome density to improve pest resistance and increase compound yields. However, two significant barriers hinder this approach: first, although several genes known to affect trichome density are now known in tomato, how they control density remains unclear, making it challenging to increase density without triggering undesirable side effects; second, the potential disadvantages of an unusually high trichome density for the plant are not well understood. This work focuses on tomato (*Solanum lycopersicon* L. cv Micro-Tom) to address these gaps by investigating the genetic and functional aspects of trichome regulation. The activity of a gene suspected to repress hair formation in snapdragons will be reduced, testing whether it serves a similar role in tomato and whether its reduced activity can increase trichome density without adverse side effects. Phylogenetic analysis has identified an orthologue of this gene in tomato, which is being investigated further through Virus-Induced Gene Silencing (ViGS). To advance this research, future studies will focus on gene editing using CRISPR/Cas9 to develop isogenic lines with varying trichome densities. These lines will enable the assessment of both the beneficial and detrimental effects associated with different trichome densities, providing deeper insights into their functional significance.

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P-3

Development of genetically edited tomatoes with reduced histamine content using the CRISPR/Cas9 system

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Keywords: tomato, CRISPR/Cas9 system, genome editing

Histamine induces an inflammatory response as a defense mechanism against external stimuli. Diamine Oxidase (DAO) is involved in the degradation of histamine, and a deficiency in DAO impairs this degradation, leading to the accumulation of histamine and the subsequent development of allergic symptoms. Although tomatoes are widely recognized for their high content of various antioxidant compounds, they also contain a measurable amount of histamine, which can be problematic for individuals with DAO deficiency and for infants. In this study, we aimed to develop tomato plants with reduced histamine content by modulating the expression of *Histidine decarboxylase 1* Gene (*HDC1*) through CRISPR/Cas9-mediated genome editing. Through analysis of gene expression in tomato plants, *HDC1* was identified as the most highly expressed gene related to histamine biosynthesis. Using the Virus-induced Gene Silencing system, we confirmed that silencing *HDC1* significantly reduced histamine content. Subsequently, gene-edited tomato plants targeting *HDC1* were developed using the CRISPR/Cas9 vector system. These gene-edited mutant lines exhibited a significant reduction in histamine content compared to non-transgenic tomato plants.

P-4

Identification of a genomic region governing monogermy in sugar beet

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Keywords: *Beta vulgaris*, bulk segregant analysis, candidate genes, flowering

In *Beta vulgaris*, two or more flowers occur as fused clusters to produce multigerm seedballs. It is undesired, hence monogermy has been introduced to modern sugar beet cultivars. To identify genome regions differentiating mono- and multigerm sugar beet plants, short read whole genome sequencing (WGS) libraries were used. They were produced from five pairs of bulk samples comprising monogerm and multigerm plants of the same segregating F2 populations obtained from crossing a defined monogerm line and different multigerm lines. The WGS reads were mapped to the EL10 reference genome of sugar beet to identify polymorphisms and delimit the region governing monogermy. Following quality filtering, over five million biallelic SNPs were used for the analysis. Bulks from the same F2 populations, but differing with respect to the phenotype, grouped together in PCA. F_{ST} values differentiating monogerm and multigerm bulks peaked in the distal segment of the long arm of chromosome 4, pointing at a single QTL governing the trait. The QTL spanned a 217 Kb-long region encompassing 20 genes. In order to develop a simple and inexpensive genotyping assay, structural variants (SVs) were identified in the region. PCR primers flanking the SVs were designed and co-segregation of the variants with the phenotype was verified. One of the investigated polymorphisms localized within the QTL, a 135 bp-long indel, was converted into a molecular marker named SCAR-J which reliably differentiated monogerm and multigerm plants. Thirty eight of 40 monogerm plants were homozygous, carrying the reference allele, while 36 of 40 multigerm plants were either homozygous carrying the alternative allele or heterozygous, which is in line with the fact that monogermy is recessive. It remains to be tested which of the 20 genes in the QTL region is the most likely candidate gene governing monogermy. To this end, we are going to determine the expression of those genes in developing inflorescences using RNAseq.

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P-5

Genetic dissection of bentazone tolerance loci in cultivated soybean: A genome-wide assication study

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Keywords: soybean, genome wide association study, bentazone

Weeds alone cause a 37% loss in attainable soybean production. Bentazone is a postemergence herbicide used to control broadleaf weeds in the cultivation of cereals, legumes, vegetables, and tuber crops. This study identified bentazone-toleThis study aimed to screen the Korean collections of cultivated soybeans in relation to bentazone, identifying the genetic loci controlling bentazone reactions to cultivated soybean collections using a genome-wide association study (GWAS). rant and bentazone-sensitive soybean germplasms from 418 cultivated soybean germplasms and found that moderate bentazone tolerance predominated in the Korean collection of cultivated soybeans. The GWAS revealed that 42 SNPs distributed on chromosomes 3, 5, 6, 13, and 20 were strongly associated with the bentazone reaction in 418 cultivated soybean accessions over three years. Of these loci, a genomic region on chromosome 5 contained significant SNPs and was identified as being involved in the bentazone reaction in both 2020 and 2021, based on FarmCPU analysis. By conducting a haplotype analysis, this study identified five putative genes, namely, *Glyma.05g145000* (ATP-binding cassette transporter), *Glyma.05g145100* (unknown), *Glyma.05g145200* (ankyrin repeat family protein), *Glyma.05g145300* (transmembrane amino acid transporter protein), and *Glyma.05g145400* (unknown). Further studies are required to confirm the involvement of the putative genes in the bentazone reaction by comparing their expression levels between bentazone-tolerant and bentazone-sensitive plants. Therefore, the results of this study can be used for marker-assisted selection in programs for the breeding of herbicide-tolerant soybeans.

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P-6

Application of wheat × barley intergeneric hybrids in genome editing

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Keywords: genome editing, CRISPR/Cas9, wheat-barley intergeneric hybrid

CRISPR/Cas (Clustered regularly interspaced short palindromic repeat/CRISPR-associated protein) technology provides a versatile, easy and inexpensive solution for precise, sequence specific *in vivo* modification of DNA including targeted mutagenesis, gene inactivation and gene/allele replacement. In the case of bread wheat (*Triticum aestivum* L.), specific inactivation of various genes has also been used to develop economically useful traits. To maintain phenotypes the constant presence of transgenes is not required, but to induce them a temporary transgenic state is needed, during which the genes encoding CRISPR/Cas are permanently expressed in the plant. Following the implementation of the modifications, the incorporated DNA is removed in subsequent generations through segregation. However, during gene transfer, smaller DNA segments can be incorporated into chromosomes, which are difficult and costly to identify or may even create unwanted traits. These events may raise concerns regarding the technology's reputation, and therefore various transient techniques that do not require DNA integration have been developed, like biolistic delivery of guideRNA/Cas ribonucleoprotein (RNP) complex into tissue cultures, but their mutational efficiency is very low. Our research group has created a genome editing system in wheat which utilizes the advantages of the wheat × barley (*Hordeum vulgare* L.) distant cross. In our strategy, the paternal barley genome is used to carry the CRISPR/Cas system, and following the wheat genome editing in the hybrid, the transgenic barley genome is removed from the offspring by back-crossing with wheat, and the wheat genome is restored to a diploid form. Our preliminary results demonstrate

that this system, due to the long-term transgene expression, is much more effective in traceless genome editing than the technologies that already exist in cereals.

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P-8

Increasing the efficiency of directed gene-specific mutagenesis by heat and chromatin modification of de-differentiated maize cells

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Keywords: maize, CRISPR/Cas9, NGS, heat treatment, chromatin modification, Na-But, NAA, AZA

Maize, the world's most widely grown crop, requires new high-yield, resilient varieties to address population growth, climate change, and rising agricultural demands. Adaptation to weather, soil types, and their nutrient content starts at the genomic level. Precision breeding using CRISPR/Cas9 is a key tool for sustainable agriculture.

In our experiments, we focused on the *ARE1* (*abnormal cytokinin response1* or *abc1*) gene, which plays a role in nitrogen use, a critical limiting factor for crop productivity. Therefore, there is an ongoing need to breed varieties with improved nitrogen utilization efficiency. To understand the role of this gene in maize, mutants were generated using CRISPR/Cas9. After designing the guide RNAs and generating the constructs, the maize line SZ17 was transformed with the construct using *Agrobacterium*-mediated transformation. The effects of different heat treatments and their durations (37°C for 48 hours or 45°C for 3 hours), as well as chromatin modifiers with histone deacetylase inhibitors (sodium butyrate_Na-But, nicotinamide_NAA), and a DNA methyltransferase inhibitor (azacytidine_AZA), were investigated in transformed calli. The effects of these treatments were analyzed by NGS in the region of the target gRNA. It was found that the 37°C for 48 hours heat treatment alone resulted in a significant increase in editing efficiency (up to 99,3%) compared to the 45°C for 3 hours heat treatment, in terms of the quantifiable deletion percentage of treated callus in the default state. The largest increase in editing efficiency was achieved with the 48-hour heat treatment at 37°C in combination with chromatin modifiers. In each of the Na-But, NAA, or AZA treatments, an increase in editing efficiency of 7–15-fold change was achieved when combined with the preliminary 37°C heat treatment, whereas only a 1.2–3.3-fold increase was observed when chromatin relaxants were used alone. It was also observed that the treatments not only increased the frequency of gene editing events but also affected the length of detectable fragment deletions. The preliminary results provide a basis for further development of technology, increasing its routine applicability and enhancing the precision breeding of agronomically important genes.

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P-9

uORF-targeted genome editing for lycopene enhancement in tomato

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Keywords: uORF, CRISPR/Cas9 genome editing, Lycopene biosynthesis, dual-luciferase assay, tomato

Lycopene, a major antioxidant pigment in tomato (*Solanum lycopersicum*) fruits, is a key target in metabolic crop improvement. Although upstream open reading frames (uORFs) located in the 5′-untranslated region (5′-UTR) are known to regulate translation of primary open reading frames (pORFs), their specific roles in carotenoid biosynthesis genes in tomato remain underexplored. In this study, we identified three putative uORFs within the 5′-UTR of the lycopene-related gene *LR2*. Dual-luciferase reporter assays revealed that these uORFs significantly repress downstream translation. To enhance LR2

expression, we employed a CRISPR/Cas9 genome editing strategy using dual guide RNAs to induce a large deletion encompassing all three uORFs. Transgenic tomato lines with the targeted uORF deletion were generated, providing a foundation for further investigation into the regulatory function of uORFs in carotenoid biosynthesis. Our findings suggest a new, transgene-free strategy for enhancing protein production through modulation of translational efficiency. Future comprehensive phenotypic and metabolic characterization of the edited lines will contribute to clarifying their potential for nutritional enhancement.

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P-10

Towards genome editing strategies in olive

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Keywords: NGTs, protoplast regeneration, *in vitro* culture

Modern biotechnological approaches have successfully been extended to a growing range of species, including woody plants, which have shown low responsiveness to *in vitro* propagation and transformation techniques compared to the herbaceous ones.

This work explores different approaches for applying New Genomics Techniques (NGTs) in olive (*Olea europaea* L.), with preliminary findings suggesting the applicability of these tools, though the development and optimization of the protocols are still necessary. The well-known recalcitrance of olive tissues to *in vitro* manipulation and regeneration of new plants represents the main obstacle to the implementation of NGTs in this species. To overcome these constraints, several methods are under investigation in our lab, including: i) the optimization of embryogenic callus culture and plant regeneration protocols specific for different genotypes; ii) the design of methods for obtaining protoplasts to be used as starting material for transfection with CRISPR/Cas9 editing agents, employing a transient delivery system based on ribonucleoproteins (RNP); iii) the plant gene editing through *de novo* meristem induction, using a Golden Gate-assembled vector with a CRISPR/Cas9 system. This multi-approach strategy will maximize the possibility of developing an efficient precision gene editing procedure in olive. Further efforts will be focused on enhancing the stability of CRISPR/Cas9 delivery systems, minimizing off-target effects, and optimizing the conditions for boosting the embryogenic competence of the cultures. As progress is made, it is anticipated that these advanced techniques will significantly accelerate the genetic improvement of olive cultivars with desirable traits useful for addressing current challenges, such as climate change-associated stresses and impact of pathogens. In particular, the developed methodologies will be applied to generate olive genotypes with increased tolerance to *Xylella fastidiosa*, a quarantine pathogen listed as one of the major phytosanitary threats throughout the Mediterranean region.

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P-11

Comparison of different gene editing methods for the introduction of targeted point mutations into corn (*Zea mays*) cell cultures

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Keywords: maize, CRISPR/Cas9, oligonucleotide-directed mutagenesis (ODM), herbicide resistance, targeted gene editing, cell cycle synchronization, chromatin modification

The development of high-performing, stress-resistant crop varieties is crucial to address the challenges posed by climate change and the growing global population. Gene editing methods represent powerful complement to traditional plant breeding techniques.

In this study, we compared two gene editing methods for the introduction of targeted point mutations into maize cell cultures: Oligonucleotide Directed Mutagenesis (ODM) and CRISPR/Cas9 system combined with template DNA.

Our target gene was the acetyl-CoA carboxylase (ACC) gene, whose targeted point mutation leads to herbicide

tolerance, selectable with cycloxydim. To enhance gene editing efficiency, SZ17 (capable of regeneration) and H1233 (more suitable for transformation) maize cell lines were pretreated with hydroxyurea alone to synchronize cell cycle or in combination with sodium butyrate or nicotinamide to relax chromatin structure. Editing molecules–oligonucleotide templates alone or together with CRISPR/Cas9 system coding plasmids–were delivered into maize cells *via* biolistic transformation.

Selection of herbicide-resistant calli was carried out on cycloxydim-containing medium, followed by isolation, PCR amplification and sequence analysis of the targeted DNA region. Herbicide-resistant calli were successfully obtained with both methods, but the desired point mutation could not be detected. One possible reason for this is the low efficiency of the biolistic transformation, therefore the above experiments are being repeated in combination with other DNA delivery methods.

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AQUATIC PLANT BIOLOGY AND ALGAL BIOTECHNOLOGY

P-12

Exogenous abscisic acid alters the growth, turion yield and biochemical composition of four ecotypes of giant duckweed, *Spirodela polyrhiza* (L.) Schleiden

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Keywords: duckweed, abscisic acid, turion yield, relative growth rate, starch, protein

Giant duckweed *(Spirodela polyrhiza* (L.) Schleiden, a free-floating aquatic plant species, reproduces mainly vegetatively through rapid production of their active and leaf-like fronds and thus colonize quickly freshwater surfaces. Under adverse environment this species is able to switch this normal developmental program and produces dormant propagules (turions) instead of active fronds as a stress avoidance strategy. It is assumed that abscisic acid (ABA) is the main internal driver of developmental switch in *Sp. polyrhiza* in stressful environment.

In this work we investigated growth and biochemical responses of four clones of *Sp. polyrhiza* to exogenously applied ABA (0-1 µM) in the growth medium over 7-days-long experimental periods. The four clones (UD0401, UD0402, UD0407, UD0408) were selected from the Duckweed Clone Collection of Department of Botany, University of Debrecen. They exhibit differences in turion-forming capacity e.g., in senescing nutrient-limited axenic cultures clones UD0401 and UD0407 produce turions, while clones UD0402 and UD0408 not.

We observed similar growth trends of the four clones under ABA treatments. Each clone showed progressive decrease in relative growth rate (RGR) of frond number and total frond area with increasing ABA concentration. Dry matter content of fronds (DMC%) changed inversely and exhibited a sharp increase with increasing ABA concentrations. ABA initiated turion formation at 0.25 µM in cultures of each clone. Specific turion yield reached the maximum at 0.5 µM ABA. Increase of frond DMC% was mostly due to starch accumulation and less by an increase in protein content. Starch accumulation was higher in turions than in fronds across all ABA treatments. Additionally, ABA increased the protein content per fresh mass (in % f.m.) in fronds of each clone, but in clone- and concentration-dependent manner. Our results provide further evidence on potential involvement of ABA in changes of developmental program of *Sp. polyrhiza.* In the studied clones, these changes appeared with a simultaneous alteration in starch and protein content in the fronds and turions.

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P-13

A sustainable approach to mitigating emerging pollutant toxicity and enhancing plant resistance to abiotic stress

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Keywords: algal biotechnology for sustainability, low-impact pollutant mitigation, circular bioeconomy

In recent years, algae have gained significant attention from the scientific community as a sustainable and promising source of various bioactive compounds. When efficiently extracted and processed, these compounds have the potential to transform unwanted algal biomass, typically considered waste, into valuable resources for a wide range of applications. Due to their high carbon content, algae are considered a viable feedstock for the production of carbon-based alternatives through innovative thermochemical processes. One of the most promising thermochemical treatments for algal biomass valorization is hydrothermal carbonization (HTC). The solid product, known as hydrochar, is a carbon-rich material enriched with oxygenated functional groups, which confer high adsorptive properties. Given the growing concerns regarding water pollution and the need for sustainable remediation strategies, hydrochar derived from algal biomass represents a promising candidate for environmental applications, particularly as a biofilter to adsorb and mitigate the toxicity of Thiram, an emerging xenobiotic widely present in aquatic and terrestrial ecosystems. In parallel, the liquid fraction derived from the HTC process (AHL) was evaluated for its effectiveness as a plant biostimulant. An experimental plan was developed to assess whether seed-priming with AHL obtained from macroalgae could enhance plant tolerance under different abiotic stress conditions. This integrated approach aims to fully valorize algal biomass within a circular economy framework, while promoting sustainable solutions for environmental remediation and crop resilience.

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P-14

Identification and characterization of a heat shock transcription factor (HSF) in the marine red alga *Pyropia yezoensis* (Rhodophyta)

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Keywords: heat shock transcription factor, PyHSF, *Pyropia yezoensis*, red algae

Heat shock transcription factors (HSFs) play a pivotal role in the high-temperature response and are found in all organisms. Terrestrial plants in particularly possess a greater number of HSF genes, suggesting their necessity for adaptation to severe dry and extreme temperature environments. *Pyropia yezoensis* inhabit intertidal zones subject to periodic desiccation and extreme temperature changes. Despite this, HSF genes have not yet been reported in red algae. In this study, we identify an HSF gene, *PyHSF,* from the marine red algae *P. yezoensis*. PyHSF has a DNA binding domain, oligomerization domain, and motifs that are well-conserved in the HSFA family of angiosperms. Phylogenic analyses showed that HSFs from red algae were grouped into a distinctive clade separate from those of green plants. PyHSF is located in the nucleus. When the *PyHSF* gene was overexpressed in single-cell green algae, *Chlamydomonas*, the transcription levels of heat response genes including heat shock proteins increased under normal growth conditions without heat stress, demonstrating that PyHSF acts as a transcriptional activator of these heat response genes. Transgenic *Chlamydomonas* cells overexpressing *PyHSF* showed a higher growth rate compared to wild-type cells under heat-stress conditions. *PyHSF* responds to high temperatures and shows different expression patterns depending on the life cycle stage of *P. yezoensis*. These results suggest that *PyHSF* has distinctive sequences those of green plants and plays an important role in regulating the growth and life cycle of *P. yezoensis*, which are highly affected by temperature changes.

P-15

Stress effect of surfactants on the *Lemna minor*

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Keywords: detergent, stress, *Lemna minor*

Nowadays, due to the increasing number of dry periods, irrigation with grey water is becoming a common practice worldwide. None of the traditional wastewater treatment methods can completely filter detergent components from wastewater. They can be detected in living waters, causing a number of environmental problems, as they can be stress factors for the ecosystem.

The aim of our research is to investigate the effects of synthetic detergents on aquatic organisms using realistic solution concentrations (concentrations 10-100 times thinner than the recommended concentration for washing (0.50-0.025 ml l^{-1})). We compared the effect of four commercially available liquid washing gels (environmentally friendly and non-environmentally friendly) and conventional laundry soap on the growth, reproduction, weight, protein content, chlorophyll and carotene content of the *Lemna minor* test organism, and the enzyme activity of peroxidase.

We found that the stress caused by detergents varied between detergents and properties.

Synthetic detergents resulted in the death of living organisms in the most concentrated dilution (0.50 – 0.25 ml l^{-1}) examined. In contrast, even at this concentration, the laundry soap solution resulted in a reproduction similar to that of the control plants. The dry weight and protein content of the plants decreased significantly in the case of synthetic detergents, while the laundry soap solution did not significantly reduce it at any of the concentrations.

The development of photosynthetic pigments (kl-a, kl-b, car) was not significantly inhibited by the 0.050 – 0.255 ml l^{-1} concentration, but the 0.10 – 0.05 ml l^{-1} concentration was already significantly inhibited. A significant increase in the enzyme guaiacol peroxidase, which plays a role in the elimination of reactive oxygen derivatives, was observed in the concentration of 0.10 – 0.50 ml l^{-1} in each detergent. (However, the death of the plants was confirmed by the cessation of enzyme activity/low rate in the most concentrated concentrations of synthetic detergents studied.)

The eco-friendly detergent did not cause significantly less stress to the plants than its synthetic counterparts. Commercially available detergents did not cause stress to *Lemna minor* at low (0.025 ml l^{-1}) solution concentrations, but at higher solution concentrations (0.10 – 0.05 ml l^{-1}) they caused significant stress to plants, which degrades our natural environment with the change in the aquatic ecological balance. On the other hand, the laundry soap solution did not reduce the physiological parameters of the plants even at the most concentrated concentration (0.50 – 0.25 ml l^{-1}).

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P-16

Promising perspectives on the exploitation of dairy effluent as a substrate for duckweed biomass production

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Keywords: dairy wastewater, duckweed, circular economy, biomass production

The escalating global water scarcity and the increasing discharge of anthropogenic wastes and effluents necessitate the urgent development of low-carbon, resource-recycling waste treatment technologies that minimize energy consumption and align with biorefinery and circular economy principles. The dairy industry, a major contributor to food processing wastewater (WW) generation, produces nutrient-rich effluents containing recoverable resources suitable for aquatic plant cultivation. Duckweeds (Lemnaceae), the fastest-growing flowering aquatic plants, emerge as a promising solution for treating dairy wastewater. Their rapid growth, efficient nutrient absorption, adaptability, and simple harvesting process allow them to purify water while simultaneously producing valuable protein-rich biomass.

This dual benefit aligns with circular economy principles, making Lemnaceae integration a sustainable strategy for the dairy sector, transforming and valorizing dairy WW into high-biomass yields and providing a sustainable source for extracting valuable compounds like pigments or biostimulants.

Initial investigations explored the cultivation of diverse duckweed species and genotypes in wastewater sourced from various cheese production lines at Santangiolina company (Pandino, Cremona, Italy). Subsequently, we conducted a lab-scale comparative study using selected clones of *Lemna minor* and *Lemna × mediterranea*. This experiment assessed the duckweed clones' growth responses to specific dairy wastewater samples by monitoring growth parameters and biochemical and physiological performance and simultaneously measuring carbon, nitrogen, and phosphorus removal. Preliminary results highlighted a trade-off: *L. × mediterranea* showed superior biomass increase, but *L. minor* demonstrated greater tolerance to the wastewater conditions. Further analyses are in progress to characterize *Lemna minor* and *Lemna × mediterranea* biomass composition. Future studies should prioritize evaluating the feasibility of selected duckweed cultivation for dairy wastewater treatment in pilot-scale or *in situ* systems.

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P-17

Microalgae-based approaches for soil health improvement, crop growth, and climate mitigation

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Keywords: soil health, carbon sequestration, biochar, microalgae biomass, CO_2 capture, sustainable agriculture, environmental benefits

Microalgae, as photosynthetic organisms, play a crucial role in carbon capture by efficiently converting atmospheric CO_2 into biomass, offering a sustainable pathway for mitigating climate change. Beyond their potential for industrial carbon sequestration, when applied to soil as biofertilizers, organic amendment, or biostimulants, microalgae enhance soil nutrient availability, promote microbial activity, and improve soil structure while reducing reliance on synthetic inputs. Moreover, the thermochemical conversion of microalgal residues into biochar provides an effective strategy for long-term carbon storage, improving soil structure, nutrient retention, and pollutant remediation. Microalgae biochar exhibits a high carbon content with strong CO_2 adsorption capacity, making it a promising material for carbon sequestration and sustainable soil amendments. While the roles of microalgae in soil fertility, CO_2 removal, and bioremediation have been studied separately, there is limited research on integrating these processes. In this paper we discussed microalgae-based soil fertility, CO_2 capture, and bioremediation. It also examines the opportunities, challenges, and environmental sustainability of these processes. By advancing the use of microalgae in soil health improvement, carbon sequestration, and bioremediation, these approaches contribute to environmental sustainability and help mitigate global environmental challenges.

P-18

Interactive effects of polystyrene micro- and nanoplastics and trace elements on oxidative stress and central metabolism in *Lemna perpusilla*

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Keywords: *Lemna perpusilla*, microplastics, nanoplastics, trace elements, oxidative stress, metabolomics, aquatic toxicology, TCA cycle

The ubiquity of microplastics (MPs) and nanoplastics (NPs) in freshwater ecosystems presents a growing ecological concern, particularly due to their potential synergistic interactions with trace elements. This study investigates the combined effects of polystyrene microplastics (PS-MPs) and nanoplastics (PS-NPs), alongside potassium sulfate (K_2SO_4) and cobalt chloride ($CoCl_2$), on the aquatic plant *Lemna perpusilla*. Plants were exposed to PS-MPs (2.5–40 mg/L) and PS-NPs (1.5–10 mg/L), either alone or in combination with K_2SO_4 (3 mg/L) and $CoCl_2$ (2 mg/L). Growth analysis revealed that both trace elements significantly promoted biomass accumulation ($p < 0.05$) compared to untreated controls. PS-NPs, owing to their nanoscale dimensions (~100 nm), demonstrated greater potential for bioaccumulation and induced higher levels of oxidative stress than PS-MPs at equivalent doses.

Biochemical and metabolomic profiling indicated that co-exposure to plastic particles and trace elements significantly modulated key metabolic pathways, including pyruvate metabolism, carbon fixation, the glyoxylate and dicarboxylate cycle, and the tricarboxylic acid (TCA) cycle. These findings underscore the complex and synergistic impacts of emerging plastic pollutants and trace elements on aquatic plant physiology and biochemistry, providing insights into pollutant interactions and their ecological implications in freshwater systems.

P-19

Unveiling molecular insights into the role of *Raphidocelis subcapitata* in enhancing essential fatty acids for biofuel production under nitrate limitations

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Keywords: Raspberry Pi, transcriptomic, microalgae

The significance of biofuels as a sustainable energy source has gained considerable attention in recent years. However, the optimization of their production is affected by numerous abiotic factors that prevent the process from achieving maximum efficiency. In this field, several studies have demonstrated the ability of microalgae such as *R. subcapitata* to increase the production of essential fatty acids in the production of biofuels under nitrogen-limiting conditions. At the same time, it has been shown that photoperiod and diurnal harvesting time point of the culture can affect the accumulation of various metabolites in these organisms. Despite the potential impact of these factors on biotechnological applications, the molecular mechanisms underlying the observed variations remain largely unexplored.

In this research we focused on determining the molecular factors involved in this physiological response by studying the transcriptomic and phenotypic behavior of *R. subcapitata* cultures subjected to different conditions of nitrate availability and photoperiod as well as different diurnal time points. All these cultures were monitored by a system of photobioreactors automated by our research group using Raspberry pi devices. For this purpose, a coupled RNAseq and fatty acid profile analysis was performed under the described conditions. The analysis of the results allowed us to establish that fatty acid profiles are rhythmic, and the photoperiods affect this rhythmicity, while nitrate limitation determines the type and total amount of fatty acids generated. In addition, a gene co-expression network was constructed from transcriptomic data to determine the genes involved in the fatty acid metabolic pathway and the transcription factors controlling this pathway. This knowledge will facilitate the optimisation of culture conditions and will lay the basis for incorporating AI in the future to further improve culture conditions for the use of microalgae in biotechnological applications such as CO_2 capture.

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P-20

Combined toxicity of silver nanoparticles and microplastics on the aquatic plant *Lemna minor*

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Keywords: duckweed, growth, microplastics, oxidative stress, silver nanoparticles

Silver nanoparticles (AgNPs) and microplastics (MPs) are known pollutants in aquatic environments, but knowledge of their combined effects on aquatic plants is scarce. In this study, the individual toxicity of AgNPs coated with polyvinylpyrrolidone (PVP) or cetyltrimethylammonium bromide (CTAB) and MPs made of polystyrene (PS-MPs) or polymethyl methacrylate (PMMA-MPs) as well as their combined toxicity was evaluated on *Lemna minor* plants. AgNP-CTAB was less toxic than AgNP-PVP as the concentrations required to reduce the 7-day growth by 50% were 1 mg/L and 0.1 mg/L, respectively. On the other hand, MPs in the range of 10-100 mg/L did not affect the duckweed growth. The combination of 0.1 mg/L AgNP-PVP with both types of

MPs (10 mg/L) showed an even stronger negative effect on the growth, whereas the combined treatments of 1 mg/L AgNP-CTAB and MPs (50 mg/L) resulted in an alleviating effect. Hydrogen peroxide content increased in plants exposed to AgNP-CTAB alone, but was reduced in the combined treatments with both types of MPs. This reduction may be related to the elevated peroxidase and catalase activities observed in the combined treatments. In contrast, AgNP-PVP applied individually did not elevate the hydrogen peroxide content, while in combination with PMMA-MPs it led to an increase. However, peroxidase and catalase activities were increased in both individual AgNP-PVP and combined treatments. These results indicate that the effects of AgNPs, whether applied individually or in combination with MPs, strongly depend on the type of AgNP coating as well as the polymer composition of the MPs.

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BENEFICIAL PLANT-MICROBE INTERACTIONS

P-21

Molecular mechanisms of fungal-mediated cadmium stress alleviation in flax: A multi-omics approach

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Keywords: cadmium stress, plant-microbe interaction, molecular analysis

Endophytic microorganisms provide a low-cost, eco-friendly way to boost food production and reduce abiotic stress, particularly Cd toxicity. They establish mutualistic relationships with host plants, colonizing intercellular and intracellular spaces without causing significant morphological changes. We examined flax's reaction to an endophytic fungus under different Cd ion concentrations. The fungus lowered Cd toxicity, restoring inoculated plant growth to levels comparable to unexposed controls. We conducted a multifaceted analysis encompassing plant physiology, proteomics, metabolomics, ionomics, hormone profiling, EDX, and electron microscopy to uncover molecular mechanisms. This integrated approach revealed detailed molecular insights and highlighted specific pathways contributing to the enhanced resilience of flax plants grown under Cd stress in the presence of the endophytic fungus. The proteomic analysis highlighted significant changes in several crucial metabolic processes, including the biosynthesis of secondary metabolites and proteasome composition, indicating an active fungal influence on plant stress responses. Furthermore, we detected protein changes associated with nutrient deficiency, suggesting that the fungus influences nutrient homeostasis. Subsequent elemental analysis using ICP-MS revealed a significant increase in the concentration of essential elements in inoculated plants. EDX measurements

coupled with electron microscopy enabled the visualization and localization of these elemental changes within distinct root tissues. The metabolomic analysis validated our findings, confirming increased secondary metabolites involved in nutrient uptake. Our findings show that endophytic fungus promotes host plant resistance to Cd stress by altering the proteome and metabolome. This change enhances nutrient acquisition and plant metabolism under Cd, reducing its adverse effects. Thus, the fungus enhances the plant's resilience instead of merely filtering Cd ions.

P-22

Application of ß-cyclocitric acid affects mycorrhizal colonization and response at the molecular level in tomato plants

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Keywords: Apocarotenoid, AMF, drought

Apocarotenoids are metabolites produced by plants and include phytohormones, signalling molecules, pigments, and volatiles. These compounds are derived from the oxidative breakdown of carotenoids and are known to modulate plant molecular and biochemical responses to overcome the adverse effects of abiotic stresses. Also, a role for apocarotenoids in arbuscular mycorrhizal (AM) symbiosis establishment has been recently documented. Particularly, the apocarotenoids β-cyclocitral (β-CC) and β-cyclocitric acid (β-CCA) are promising compounds reported to promote plant tolerance to drought. However, their effects on mycorrhizal colonization and plant-microorganism interaction under abiotic stresses are still largely unknown. This study investigates the effects of exogenous application of β-CCA on AM colonization and AM-inoculated tomato plant response under a water deficit condition.

In detail, tomato plants (cv. San Marzano Nano), inoculated with *Funneliformis mosseae* (myc) or not inoculated (non myc), were treated with β-CCA (supplied through irrigation water) or just irrigated with regular water. Two-months-old plants were subjected to water deficit stress (WS) or regularly watered (WW). Throughout the whole trial, physiological and biometric data were recorded. After harvesting, roots were split in half to assess AM fungal colonization and to perform RNAseq analysis, which was also carried out on shoot samples. Results showed that β-CCA enhanced the frequency of AM fungal colonization under WS without affecting the percentage of arbuscules. Transcriptomics analysis revealed that β-CCA led to an up-regulation of genes involved in antioxidant responses, such as genes coding for heat shock proteins and scavenging enzymes. Under WS, myc plants treated with β-CCA showed the greatest transcriptional reprogramming both in shoots and roots, with up-regulation of genes putatively related to stress tolerance. Our findings show that application of β-CCA could support mycorrhizal symbiosis under water deficit conditions, while inducing the regulation of genes related to drought tolerance. This supports β-CCA as a suitable compound for sustainable agriculture, able to improve plant stress response at molecular level without compromising beneficial microbial association.

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P-23

Silicon: A key modulator of nodule metabolism in *Trifolium incarnatum*

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Keywords: biostimulant, dinitrogen fixation, proteomic

Silicon (Si) is known to alleviate biotic and abiotic stresses in plants, but its role in legume nodulation and nitrogen fixation remains poorly understood. This study investigates the impact of Si supply on the physiological traits and nodule functioning of *Trifolium incarnatum* inoculated with *Rhizobium leguminosarum bv trifolii*. Plants were grown under hydroponic conditions with or without Si supplementation (1.7 mM Na_2SiO_3) for 25 days. Si supply significantly enhanced shoot biomass, total nitrogen content, and nodule development. Nodules from Si-treated plants exhibited increased numbers, biomass, and density, as well as a higher nitrogenase abundance, leading to improved nitrogen fixation efficiency. Ionomic analysis revealed that Si supply increased the accumulation of key macroelements (K, P, S) and microelements (Cu, Zn, Mo) in nodules, which are known to enhance nodulation efficiency. Proteomic analysis identified 1,200 differentially accumulated proteins (DAPs) in nodules, showing Si-induced

modulations in both plant and bacterial symbionts. Several DAPs were linked to nitrogen metabolism and symbiosome function, suggesting a direct role of Si in optimizing nodule activity. These findings provide new insights into the beneficial effects of Si on legume nodulation and nitrogen fixation, with potential implications for sustainable agriculture.

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P-24

Analyzing the cell cycle inhibitor RETINOBLASTOMA-RELATED protein in *Medicago truncatula* during nodule development

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Keywords: nodulation, RETINOBLASTOMA-RELATED (RBR), SCARECROW (SCR)

The development of root nodules hosting the symbiotic rhizobia is initiated from a group of cortical cells which re-entered into the mitotic cycle in response to rhizobia-released Nod factors. Maintenance of cell division capacity in nodules leads to the establishment of a persistent meristematic niche from which cells exit continuously and then enter the nodule differentiation program. Based on the current model, emergence of the nodules and nodule meristematic activity are controlled by almost the same molecular players of the root apical meristem. In root meristems, cell cycle entry is gated by the CDK (cyclin-dependent kinases)-mediated phosphorylation of the cell-cycle inhibitor RETINOBLASTOMA RELATED (RBR) protein. Inhibitory role of the RBR is based on its capacity to bind E2F transcription factors and LxCxE-domain containing proteins, such as SCARECROW (SCR), in order to maintain the cell cycle quiescence. SCR is a well-known GRASS-type transcription factor guiding the formative division in the *Arabidopsis* root meristem that gives rise to the emergence of the endodermis and cortex (ground tissue). In *M. truncatula*, SCR has been recently revealed to specify the distinct fate of cortical cells to enable nodulation. Contrary to *Arabidopsis*, *Mt*SCR was shown to express both in the cortex and endodermis, and its function was found to be required for nodule development (Dong et al., 2021). However, whether RBR is involved in the regulation of nodule development through the regulation of SCR, and whether RBR controls the meristematic activity in the nodules, is not yet known. In our project we attempt to characterize RBR in *M. truncatula* in general and reveal its contribution to the SCR-mediated nodule formation pathway.

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Dong, Wentao, et al. "An SHR–SCR module specifies legume cortical cell fate to enable nodulation." Nature 589.7843 (2021): 586-590.

P-25

Genome-wide identification and expression profiling of ammonium and nitrate transporters in *Medicago truncatula* under nitrogen-fixing symbiotic conditions

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Keywords: symbiotic nitrogen fixation, ammonium, nitrate

Legume crops play a crucial role in sustainable agriculture introducing nitrogen (N) to agroecosystems through symbiotic nitrogen fixation. However, high N levels in soils rapidly suppress the establishment and functioning of this symbiosis, limiting their contribution to soil fertility. To reduce the dependency on chemical fertilizers and optimize organic amendments management, it is essential to understand how legumes perceive and respond to different N sources. Nitrate and ammonium, the two main inorganic nitrogen sources in soils, activate distinct signaling pathways that regulate plant development, including the establishment of legume-rhizobium interactions. However, the molecular basis for these regulatory processes is just starting to emerge.

Nitrogen uptake depends on different transport protein families, namely, ammonium transporters (AMTs) and nitrate transporters (NPF, previously named the NRT1/PTR, and NRT2 families). In contrast to the advances in the identification of gene AMT and NPF gene members in *A. thaliana*, a comprehensive genome-wide analysis has not been carried out in legume plants.

In this work our goal is two-fold: 1) to define gene members of the AMT and NPF families in the model legume *Medicago truncatula*; 2) to identify transcriptional changes occurring in these gene families during both the initiation of the symbiosis and in response to nitrate or ammonium application once nitrogen fixation has been established. For the first objective, we used the latest *M. truncatula* genome version and carried out reciprocal BLAST protein queries combined with phylogenetic analysis. For the second objective, we queried public transcriptomic databases covering both roots undergoing rhizobium infection and mature nitrogen-fixing nodules.

This approach will provide new insights into the regulatory mechanisms controlling nitrogen fixation under varying N availability, improving our understanding of how legumes integrate N signals to optimize symbiotic nitrogen fixation and enhance agricultural sustainability.

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P-26

A reclamation project on dredged mud from Lake Balaton: Seasonal changes in the physiology of Szarvasi-1 energy grass and the connected bacterial community composition in the rhizosphere

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Dredging is necessary to maintain healthy water ecosystems and waterways, but the deposits of dredged mud are considered waste because it may contain contaminants, however, it contains a lot of nutrients too. One possibility of its utilization is growing biomass plants on it for green energy production. Within the framework of this research we aimed to optimize the cultivation of Szarvasi-1 energy grass (Elymus elongatus subsp. ponticus cv. Szarvasi-1) on dredged mud from lake Balaton.

The experiment was conducted outdoors, fifty 1 m^3 containers were filled with dredged mud or garden soil (used as control) and energy grass seeds were sawn on it. Nine different treatments were applied to optimise the biomass yield of grass: mycorrhizal inoculant (Inoq) and bacterial inoculants (AGRO Bio Hungary Ltd.) alone and combined with artificial fertilizers (VOG Export-Import Ltd.). Plant physiological parameters were collected as well as microbiological investigations of the bacterial community in the mud was performed. The growth of the energy grass was monitored monthly, and at the beginning of spring, additional physiological parameters were measured (e.g., relative chlorophyll content of leaves (Chl), photochemical reflectance index (PRI) and water content of the grass). The first harvest took place in the middle of summer. The monitoring of the plant physiological parameters was resumed after the grass started to grow again. The second harvest has been done in the middle of autumn after which the plants went into dormancy during the winter. After the two harvests the biomass yield in each container was also measured. For microbiological investigations, mud and soil samples were taken to determine the initial and later microbial community composition and visualize the effect of the growth of the plants to the community. The first samples

were also used to determine the chemical parameters of the mud. Community DNA was extracted from the samples (QIAGEN) and amplicon sequencing was performed (Illumina Novaseq 6000). The resulting sequence data were processed using the mothur program (https://mothur.org).

According to our results the organic matter and nutrient content of the mud are very high, which supports rapid growth of the grass. Most macronutrient concentrations are much higher in the mud compared to the control soil, especially calcium which is 10-fold higher. The total dry matter yield on mud was 1.5-1.9 times higher compared to control soil. The Chl did not show significant variation across the treatments and spring-summer and autumn seasons whereas PRI showed significant decline in the autumn. Water content of the plants in turn showed variation between the teatments. Based on the data obtained, bacterial inoculants performed better than other treatments.

Actinobacteriota and *Pseudomonadota* were found to be the most abundant phyla during the seasons, but representatives of the *Chloroflexi*, *Gemmatimonadota*, *Bacteroidota*, *Firmicutes*, and *Myxococcota* phyla were also present in all samples. Differences in the prokaryotic community composition could be observed as influenced by environmental factors (e.g. light exposure and connected temperature, due to the arrangement of the containers). To demonstrate the effects of the treatments further correlation analysis is needed.

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P-27

Context-dependent interaction between plant parasitic nematodes and mycorrhiza: A meta-analytical perspective and experimental approach

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Keywords: Plant-parasitic nematodes, arbuscular mycorrhizal fungi, analytical review, greenhouse experiment, biocontrol, molecular mechanisms

Plant-parasitic nematodes (PPN) represent a group of pests that significantly impair plant performance and reduce crop yields. In search for environmentally sustainable alternatives to chemical control, current research is increasingly focused on beneficial soil-borne organisms as potential biological control agents against PPN. Among these, arbuscular mycorrhizal fungi (AMF) are key root symbionts known to enhance plant nutrition and confer protection against abiotic stresses and various soil pathogens, including PPN. Nevertheless, evidence regarding the protective role of AMF in PPN-infected plants remains inconsistent, making it challenging to reliably anticipate the ecological and agricultural benefits that mycorrhizal associations might confer. The aim of our research was to elucidate the mechanisms and factors underlying plant protection against PPN in the context of AMF symbiosis. First, we performed a meta-analysis of existing literature, revealing that the biocontrol efficacy of AMF depends on both fungal identity and the life strategy of the nematode. Second, using a tomato-based model system involving the AMF *Rhizophagus irregularis* and the root-knot nematode *Meloidogyne incognita*, we assessed the performance of both root-associated organisms and the host plant under different levels of phosphorus and nitrogen availability. We observed that nutrient availability not only influenced the development of both AMF and PPN, but also modulated plant's response, as evidenced by differential expression of defense- and signaling-related genes. Together, our findings indicate that the outcomes of the tripartite interaction among

plants-AMF-PPN are highly context-dependent. Future research into the molecular and rhizosphere signaling pathways shaping the interaction will deepen our understanding the functioning and potential outcomes of these tripartite interactions to promote the rationale exploitation of AMF as biological control agents in sustainable agriculture.

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P-28

An inversion in the *Medicago truncatula dnf6* mutant affects the function of the citrate transporter *MtMATE67* resulting in defective symbiotic nitrogen fixation

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Keywords: Symbiotic nitrogen fixation, *Medicago truncatula*, MATE family

The model legume *Medicago truncatula* establishes nitrogen-fixing interaction with endosymbiotic *Sinorhizobium* sp. leading to the formation of root nodules wherein rhizobia convert atmospheric dinitrogen into ammonia. This process is crucial for agriculture because the cultivation of legumes does not require synthetic fertilizers. Even more, they increase combined nitrogen level of soil making these species essential components of sustainable agriculture. Our research focuses on a better understanding of the regulation of symbiotic nitrogen fixation by identifying and functional analysis of plant genes involved in nodule development and function. *M. truncatula* plants provide an excellent tool for discovering genes involved in biological processes, particularly through analyzing symbiotic mutants showing defects in symbiotic nitrogen fixation. In this study, we focused on the *dnf6* symbiotic mutant of *M. truncatula* generated by fast neutron bombardment. The *dnf6* mutant showed the symptoms of nitrogen starvation (yellowish leaves and retarded growth) roots under symbiotic conditions and developed small white ineffective nodules indicating the absence of leghemoglobin. In contrast, wild-type plants developed elongated pink nitrogen-fixing nodules and showed vigorous growth under symbiotic conditions.

To identify the *DNF6* gene, we carried out genetic mapping combined with whole genome sequencing of the *dnf6* mutant. The genetic mapping identified the symbiotic locus of *dnf6* on the upper part of chromosome 8. The analysis of the genome sequence of the *dnf6* mutant did not detect deletions in the region defined by genetic mapping, however, the meticulous analysis identified chimeric reads at two positions in the DNF6 region, indicating a potential inversion in the *dnf6* genome which was confirmed by PCR analysis. The inversion affected the structure of the *Chr8g0352151* gene between the eighth and ninth exons. The gene *Chr8g0352151* codes for the MATE67, a member of the Multidrug and Toxic Compounds Extrusion (MATE) family, which has been previously demonstrated to be involved in the transport of citrate. Citrate is crucial for the solubilization and transport of iron, and therefore it is essential for nodule function and nitrogen fixation. To confirm that *MtMATE67* is responsible for the *dnf6* phenotype, genetic complementation was carried out with a construct of the *MtMATE67* gene, which restored the effective nitrogen-fixing symbiosis in *dnf6* mutant indicated by the development of pink nodules displaying typical zonation of indeterminate nodules and colonization of the nitrogen fixation zone by rhizobia. These findings confirmed that the *dnf6* mutant is defective in *MtMATE67* and this gene is essential for effective symbiotic nitrogen fixation in *M. truncatula* accession Jemalong.

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P-29

'Shining chloroplasts' – temporal accumulation of nitric oxide in changing chloroplasts during protection of tomato plants against gray mold disease by application of trichoderma biocontrol agents

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Keywords: nitric oxide, chloroplast, biocontrol

Trichoderma fungi have been shown to function as biocontrol agents (BCA), with the capacity to enhance

plant resistance to biotrophic and necrotrophic pathogens. According to the findings of recent studies, some *Trichoderma* strains are able to induce TISR (*Trichoderma*-Induced Systemic Resistance) in plants, a combination of Induced Systemic Resistance (ISR)/Systemic Acquired Resistance (SAR), in which nitric oxide (NO) is proposed to play an important role.

Results of the latest research and own preliminary studies suggested the important role of *Trichoderma virens* TRS 106, obtained from the bank collection of the Department of Microbiology and Rhizosphere, Institute of Horticulture – National Research Institute, as BCA, which significantly increased tomato (S. *lycopersicum* L.) plant defence responses against *Botrytis cinerea*, slowing down the development of grey mould disease in plants. The studies presented here show the changes in plants and chloroplasts, including the accumulation of NO, in tomato plants where significant disease inhibition was observed. The study showed two-peak increase in NO accumulation in plants pretreated with *Trichoderma* spores, added to the soil, at 0-72 h after *B. cinerea* inoculation. Temporary accumulation of NO was also evident in chloroplasts. Analysis of chloroplast function, based on photosynthetic pigment content, photosynthetic parameters, including the OJIP test, and ultrastructure analysis, showed that the use of strain TRS 106 reduced the negative effects of *B. cinerea* on chlorophyll a content and the functioning of the Ps II photosystem. Furthermore, the use of *Trichoderma* fungi appeared to reduce structural changes within chloroplasts caused by *B. cinerea*. The results obtained allow concluding that the applied strain has a positive effect on the state of the photosynthetic system and chloroplasts, which may be an important element in the protection of tomato plants against grey mould. Given that NO accumulation was observed in the protected chloroplasts, further studies will be devoted to assessing the role of this molecule in *Trichoderma*-induced protection of chloroplasts and the interaction pathways of NO with other molecules involved in defence responses.

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P-30

Tomato genotype and rhizosphere microbiome can contribute to water stress tolerance

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Keywords: drought stress, plant microbiome, shotgun metagenomics

Climate change is responsible for the increased frequency of extreme events and water stress on crops. Tomato is one of the most important crops worldwide, and drought-tolerant genotypes are under development. Plant-associated microorganisms can contribute to plant growth and tolerance to abiotic stress, but scarce information is available on the possible contribution of rhizosphere microbiome in mitigating water stress in tomato plants. This study aimed to understand the role of tomato genotypes and water availability in shaping rhizosphere microbial communities and to identify microbial functions possibly implicated in plant stress mitigation. A total of 215 tomato genotypes derived from a multi-parent advanced generation inter-cross (MAGIC) population (developed by ISI Sementi, Italy) were screened under controlled conditions. Thus, 10 susceptible and 10 tolerant genotypes were selected according to wilting symptoms, shoot dry weight, and plant dry weight. Rhizosphere samples were collected from well-watered and water-stressed plants after five days of recovery, and DNA was extracted from four replicates. Shotgun Illumina sequencing and bioinformatic analyses revealed complex communities associated with tomato roots under water stress. In particular, *Streptomyces*, *Nocardioides*, *Mycobacterium* (Actinomycetota phylum) and, *Coprothermobacter*, *Sphingomonas* and *Pseudomonas* (Pseudomonadota phylum) resulted as dominant genera of tomato rhizosphere and they were possibly involved in soil nutrient cycling and organic matter degradation. Metagenomic analysis of the tomato rhizosphere will clarify the effect of tomato genotypes for selecting beneficial microbial communities and identify microbial gene functions possibly associated with water stress tolerance and plant growth promotion.

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P-31

Enhancing strawberry aroma under drought stress: The role of antarctic fungi in modulating volatile ester production

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Keywords: drought stress, ester biosynthesis, fungal endophytes

Strawberries (*Fragaria* x *ananassa*) are highly appreciated globally for their distinctive aroma and other quality characteristics. Pyruvate decarboxylase (PDC) enzyme plays a crucial role in aroma formation by converting pyruvate into acetaldehyde, a precursor for esters and aromatic compounds that contribute to the strawberry's fragrance. Additionally, alcohol acyltransferase (AATs) enzymes facilitate the transfer of acyl groups, further enhancing the diversity of fruit aromas. However, strawberries are particularly susceptible to drought, which negatively impacts product quality. A promising strategy to cope with water stress involves using plant root-associated fungi. This study examines how inoculating strawberries with Antarctic fungi affects the expression of the *FaPDC* and *FaAAT* genes, which are linked to the production of volatile organic compounds (VOCs). Results showed that fungi-inoculated plants under drought conditions exhibited significant changes in gene expression, leading to an increase in volatile ester production, especially acetate esters, which are key contributors to strawberry aroma. The findings highlight the ability of Antarctic fungi to influence the metabolic pathway responsible for volatile esters by stimulating the expression of *FaPDC* and *FaAAT* genes. This research not only deepens our understanding of the molecular mechanisms behind aromatic compound biosynthesis in fruits but also emphasizes the potential of Antarctic microorganisms as tools to preserve and enhance the sensory qualities of agricultural products under water stress conditions.

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P-32

A novel plant growth-promoting rhizobacteria (PGPR) promotes plant root growth, characterized by genome sequencing and functional analysis

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Keywords: adventitious roots, auxin, lateral roots, plant growth–promoting rhizobacteria (PGPR), *Pseudomonas*, whole-genome sequencing (WGS)

Plant growth–promoting rhizobacteria (PGPR) are soil microorganisms that generate substances that enhance plant growth. In this study, we identified a new PGPR strain in the rhizosphere of *Lycium chinense* seedlings, which produce fruit with a high protein content. Whole-genome sequencing and annotation revealed that the genome of the novel bacterial strain consists of a 6.65-Mb circular chromosome featuring 5,980 predicted protein-coding sequences. Comparative genomic analysis classified this strain within the genus *Pseudomonas*; therefore, we designated it as *Pseudomonas* sp. A-2. The A-2 genome encodes proteins

involved in the biosynthesis and signaling pathways of indole-3-acetic acid (IAA), a phytohormone essential for the promotion of plant growth. This finding was substantiated by IAA detection assays and quantitative concentration analyses. Compared with control treatment, treatment with A-2 significantly enhanced growth rates by 3-fold for *Arabidopsis*, 1.5-fold for tobacco, and 1.35-fold for peanut plants. Treatment with A-2 induced expression of IAA biosynthesis genes that are also associated with lateral and adventitious root formation pathways. In *Arabidopsis thaliana,* A-2 treatment induced the expression of key genes involved in IAA synthesis from tryptophan, such as *AMI1*, *TAA1*, and *YUCs*. Upregulated genes encoding enzymes involved in the conversion of indole-3-butyric acid to IAA, including *IBR1*, *IBR10*, and *ECH2,* were also identified as being involved in adventitious root formation. The plant growth–promoting properties of this newly identified strain suggest its potential to serve as an effective biological agent in agriculture that may be able to enhance crop yields.

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P-33

Investigating the role of endophytes in enhancing grapevine resilience to drought

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The cultivation of grapevines has largely relied on increasing water use, impacting environmental water balance. Climate change and worsening droughts have further reduced water availability, making agricultural systems more vulnerable ecologically and economically. Utilizing endophytes to enhance plant growth in both optimal and stressful conditions emerges as a sustainable approach to improve grapevine resilience to water scarcity. In this study we isolated culturable bacterial communities from leaf tissues of grapevines in Italy's arid regions where water scarcity is a pressing concern. These endophytes were carefully assessed for their plant growth-promoting traits, which include the ability to produce phytohormones, fix nitrogen, solubilize phosphates, and produce siderophores. The most promising endophytes were then combined into consortia and used to inoculate endophyte-free grapevines in controlled laboratory conditions. To evaluate the effectiveness of endophyte inoculation in boosting grapevine drought resistance, the inoculated plants were subjected to drought conditions, and various physiological parameters were measured. These parameters included leaf water potential, stomatal conductance, photosynthetic rate, and antioxidant enzyme activities. Additionally, the study examined the

endophytes' impact on the transcriptome and epigenome of the inoculated plants, providing insights into the molecular mechanisms underlying the observed drought resistance.

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BIOTECHNOLOGY AND SYNTHETIC BIOLOGY

P-34

Identification and functional analysis of High-Affinity K+ Transporters (HKTs) in wheat under salinity stress

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Keywords: genome-wide analysis, high-affinity K^+ transporters (HKT), salinity stress, gene expression

Potassium (K^+) transporters, especially high-affinity K^+ transporters (HKTs), are crucial for plant growth and responses to abiotic stress, regulating Na^+ and K^+ homeostasis to reduce sodium toxicity in plant tissues. While HKTs have been studied in various crops, their characterization and functional roles in wheat, particularly regarding adaptation to environmental stresses like salinity, remain largely unexplored. This study utilized bioinformatics tools, including web platforms and software applications, to identify, characterize, and locate *HKT* genes in the wheat genome. A greenhouse experiment assessed the RNA expression levels of key salt-responsive genes in wheat seedlings exposed to different NaCl concentrations (0, 200, and 400 mM). Genome-wide analysis identified 35 *TaHKT* genes distributed across ten chromosomes, with most localized to the plasma membrane. Promoter analysis revealed many *cis*-elements linked to hormonal and abiotic stress responses. Gene expression analysis indicated a significant upregulation of *TaHKT6* and *TaSOS1* in seedlings under 400 mM NaCl, while *TaNHX1*, *TaNHX2*, and *TaTVP1* exhibited increased expression in response to both 200 mM and 400 mM NaCl stress compared to controls. These findings enhance our understanding of the role of *TaHKT* genes in wheat's response to high salinity and establish a foundation for future research on K^+ transporters in plants.

P-35

StrigoSense: A novel versatile biosensor of strigolactone responses in plants

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Keywords: strigolactones, biosensor, plant physiology

Strigolactones (SLs) are a class of plant hormones that participate in many plant environmental interactions, including responses to drought. Mapping their spatiotemporal distribution during developmental events and stress responses is very difficult due to their very low concentrations in plant tissues. To better understand their dynamics, some genetically encoded biosensors have been developed in recent years, but they all suffer from some technical limitations: namely they are negative sensors whose signal is degraded in the presence of SLs, and they require laborious and invasive techniques for signal detection. With StrigoSense, we aim to construct a novel biosensor, which is positively activated in the presence of SLs, and whose output signal can be user-defined.

For this, we will develop a common sensor module, composed of the D2 domain of the SL-responsive protein SMXL6 fused to the antiCRISPR AcrIIA4 protein, together with a dCas9-based transcriptional activation system (dCasEV). A second module, which can be user-defined, comprises the reporter gene(s) of choice, controlled by a synthetic promoter containing the target sequence of the CRISPR-based activation system. Thus, in the absence of SLs, the antiCRISPR protein inhibits dCasEV activity, maintaining the reporter signal off. When SLs are supplied, the SL-dependent polyubiquitination of D2 leads to the concomitant degradation of the antiCRISPR protein, allowing the dCasEV system to boost reporter transcription. To ensure the functionality of both partners in the D2-AcrIIA4 fusion protein, we have developed and modelled alternative designs and tested their interaction with the dCas9 protein *in silico*. Two of them have been selected for testing *in vivo*.

The StrigoSense biosensor will be first developed with fluorescent and bioluminescent reporter systems, which allow to tackle different questions in plant biology: while fluorescence gives insight into single-cell activation events with high spatiotemporal resolution, bioluminescence can be used to track system-level activation and to screen SL-dependent responses under different experimental conditions. Importantly, the use of an autonomous bioluminescent pathway derived from the fungus *Neonothopanus nambi* circumvents the need for laborious sample manipulation and requires no substrate addition to visualize reporter signal. StrigoSense will be developed and tested in Arabidopsis and tomato, seeking to move from model organisms to crops and towards biotechnology-based agricultural solutions.

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Improvement in plant regeneration from callus-derived protoplast cultures of garlic and onion

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Keywords: *Allium sativum* L., *Allium cepa* L., DNA methylation, histone deacetylation

Despite the substantial progress made over the decades garlic and onion, like other monocotyledonous plants, are still considered to be a recalcitrant species with respect to protoplast cultures. Very often this recalcitrance manifests in the early stages of cultures with abnormalities in the reconstitution of the cell wall, which consequently prevents the re-entry of mitotic divisions. Even if the cell wall is reconstituted, the resumed mitotic activity is often stopped very quickly. Various strategies are used to stimulate protoplast re-differentiation, including isolation of protoplasts from embryogenic tissues, embedding of the cells in biopolymers matrix (*e.g.* in agarose) or supplementations with specific growth regulators such as *e.g.* phytosulfokine.

Protoplasts were isolated from embryogenic callus induced from garlic clove fragments on BDS media supplemented with 2,4-dichlorophenoxyacetic acid. The source materials were digested in the presence of different concentrations of pectinolytic and cellulolytic enzymes. Released protoplasts were embedded in agarose beads and cultured in liquid CPP media supplemented with phytosulfokine, putrescine or *N*-(2-chloro-4-pyridyl)-*N*′-phenylurea and KM media supplemented with phytosulfokine, azacytidine or SAHA.

Protoplast-derived tissue was regenerated into plants on BDS medium supplemented with 1-naphthalenacetic acid and 2-isopentenyl adenine or on ½ BDS hormone-free medium.

The presence of driselase in the enzyme mixture allowed release more protoplasts. Protoplast viability, assessed by fluorescein diacetate staining, was very high and averaged about 90%. Regardless of the medium, up to the tenth day of culture, most protoplasts increased in size and changed shape from spherical to oval, which was the morphological evidences of cell wall reconstruction. Other structural changes which usually precede the first mitotic division such as reorganization of the cytoplasm and cell organelles, also took place. Although the establishment of a cell plate accompanying cytokinesis was observed, only once development of protoplast-derived callus was recorded on the rich KM medium supplemented with phytosulfokine and azacytidine or SAHA. After about 90 days, callus overgrown agarose droplets. Transferred to solid medium proliferated and formed a proembryogenic mass which then formed somatic embryos converting into plants.

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Technological development of *in vitro* micropropagation of Jerusalem artichoke (*Helianthus tuberosus* L.)

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Keywords: Jerusalem artichoke, in vitro propagation, direct organogenesis

Jerusalem artichoke (*Helianthus tuberosus* L.) is a tuber-producing crop of North American origin, classified as a pseudoannual species. Although botanically perennial, only its underground tubers survive after each growing season. Agriculturally, it is cultivated as an annual crop and is closely related to the common sunflower (*Helianthus annuus* L.). The species is of increasing interest due to its broad agronomic and industrial potential.

Its tubers are rich in inulin, B-vitamins, flavonoids, and essential amino acids, making them valuable for human and animal nutrition. In addition, the leafy biomass, which has so far been underutilized, represents a promising feedstock for green biorefinery applications. Despite these advantages and the favorable climatic conditions in Hungary, the crop remains underexploited in local agriculture.

Jerusalem artichoke can be propagated both generatively and vegetatively. Seed-based propagation is rarely used in practice due to a high level of floral self-incompatibility, although it remains indispensable for breeding purposes. Vegetative propagation from tubers is widespread but poses phytosanitary risks, including the transmission of pathogens. Consequently, there is a growing demand for alternative propagation techniques that ensure both genetic integrity and plant health. Plant tissue culture offers a viable solution, allowing for the rapid, large-scale production of disease-free planting material.

This research focused on developing a direct organogenesis-based *in vitro* micropropagation protocol initiated from primary shoot cultures. The cultivar 'Rubik' was used as experimental material. After surface sterilization, one or two nodal segments were placed onto media containing various concentrations of 6-benzylaminopurine (BAP) and gibberellic acid (GA_3). A total of six media combinations were evaluated. Cultures were maintained for 40 days under controlled environmental conditions.

Stereomicroscopic evaluation revealed prolific shoot formation from each nodal segment. After removing the leaves, multiple developing buds were clearly visible. The highest number of shoots was recorded on medium supplemented with 0.5 $mg \cdot L^{-1}$ BAP. Meanwhile, the greatest number of atypical shoot structures occurred on medium containing 1.0 $mg \cdot L^{-1}$ BAP and 0.1 $mg \cdot L^{-1}$ GA_3.

In conclusion, our study demonstrates that *in vitro* propagation via direct organogenesis is a feasible and efficient method for Jerusalem artichoke micropropagation. The optimized conditions offer a reliable platform for producing high-quality, pathogen-free propagules. Future research will focus on rooting and acclimatization protocols, genetic stability assessments, and the potential integration of this system into breeding programs.

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Creating large-scale deletions in rhizobia using a compact Cascade-Cas3 system

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When it comes to rhizobial genome engineering, researchers in the field are consistently faced with the same problem. The number of synthetic biology tools at their dispersal is nowhere near compared to some of the more widely used bacterial species. Recent years have seen some improvement with the introduction of novel genetic engineering technologies mediated by Tn5 transposon, site-specific recombination, or even CRISPR-Cas technology, however, to our knowledge, no approach exists for rhizobia, that would enable the researcher to accurately create large scale scar-less genomic deletions [1]. Here, we would like to present a CRISPR-based system capable of creating large-scale deletions that have been tailored to work in rhizobia.

The CRISPR system is part of the bacteria's adaptive immune system that protects it against invasive nucleic acids like phages and plasmids, using an RNA-mediated interference mechanism [2,3]. Some CRISPR systems have been utilized for genome editing for over a decade now, however, the use of Cas3-based systems is a recent development [4]. Cas3 is a 3'-5' single-strand DNA helicase-nuclease, part of the Class I Type I family of CAS proteins. Unlike CAS9 or CAS12a, it degrades the target DNA processively, which makes it suitable for large-scale genomic deletions [3]. This property of Cas3 opens up new opportunities in genomics, such as the complete deletion of genes, or even entire genomic regions. Csörgő et al.(2020) devised a compact Cascade – Cas3 system with the aim of creating a method that is suitable for heterologous genome engineering in bacteria [3].

We adopted and tested this system in rhizobia by placing the Cascade-Cas3 system under the control of two *Sinorhizobium meliloti* promoters inducible by rhamnose (*pRhaR*) and taurine (*pTauA*), respectively, reported to provide tight regulation with *pRhaR* having lower basal activity while *pTauA* reaching higher levels of expression [5]. In our tests, we targeted the *ExoB* and *LpsZ* genes where mutations result in easily detectable phenotypes such as lack of EPSs (compact colony morphology and Calcofluor dark) and changes in phage sensitivity/resistance pattern, respectively.

The first results revealed that *pTauA* is leaky because some of the colonies obtained after the introduction of the plasmid with the targeting system via mating showed mutant phenotype even without induction. After taurine induction, all the randomly selected colonies showed mutant phenotype and the colony PCRs revealed the presence of deletions. However, the expression of the system from the *pRhaR* promoter was unsuccessful. Genome sequencing some of the mutant strains revealed the presence of deletions around the target sequence in the 0.3 to 146 kbp range. Controlling and limiting the size as well as the endpoints of the deletions have been attempted by providing the bacteria with homologous repair templates [3], cloned into non-replicating integrative and replicative plasmids that were conjugated into the cells before the introducing the Cascade-Cas3 system to create mutations.

The details of our method and our suggestions based on the results will be discussed.

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Comparison of in-solution, FASP, S-trap, and SP3 sample preparation methods for LC-MS/MS-based proteomic analysis of three different rice tissues

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Keywords: data-independent acquisition, proteomics, rice, timsTOF, trypsin digestion

Efficient trypsin digestion remains a major challenge in bottom-up proteomics of plant materials, directly impacting subsequent mass spectrometric analyses. Conventional workflows, such as in-solution digestion, are among the most widely utilized methods for plant sample preparation for LC-MS/MS, however, they often require extensive use of detergents, which can interfere with downstream LC-MS/MS performance. To address this limitation, filter-aided sample preparation (FASP) was developed, though concerns regarding reproducibility have been raised due to its multi-step nature. More recently, alternative methods such as suspension trapping (S-Trap) and single-pot, solid-phase-enhanced sample preparation (SP3) using carboxylated or HILIC paramagnetic beads have been introduced. In particular, the SP3 method has gained considerable attention by enabling on-bead digestion, minimizing

sample loss, improving proteome coverage, reproducibility, reducing missed cleavages, and handling time. In this study, we aimed to evaluate optimal sample preparation strategies for plant proteomic analyses across four different rice tissues. We applied four distinct methods, in-solution digestion, FASP, S-Trap, and SP3, to digest proteins obtained from rice seeds, roots, and leaves, followed by proteomic profiling using a data-independent acquisition combined with parallel accumulation–serial fragmentation (diaPASEF) approach on the timsTOF platform. As a result, a total of 2,902, 3,602, and 4,163 significant proteins were identified from rice seeds, roots, and leaves, respectively. Venn diagram analyses revealed that while a significant number of proteins were commonly detected across methods, SP3-based digestion yielded 3.2- to 18-fold more uniquely identified proteins compared to the other approaches. Moreover, samples prepared by SP3, particularly in leaf tissues, exhibited stronger and clearer peptide peaks during early MS1 retention times, along with superior reproducibility. Taken together, although each method demonstrated distinct strengths and limitations, the SP3 approach offered high reproducibility, efficient sample preparation with minimal detergent interference, and strong compatibility with small-scale plant tissue samples. Thus, SP3-based workflows are proposed as a robust and scalable platform for plant proteomics, especially when sample quantities are limited.

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Identification and characterization of biological parts in plants

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Plant synthetic biology can be broadly divided into two main research areas: circuit design research and the development of biological parts. To facilitate the precise regulation of gene expression, the development of biological parts involves the identification and development of biological components (such as promoters, terminators, signal peptides, UTRs, and leader introns) that are required in plants. This has been further enabled by recent advances in large-scale genome sequencing, integrative omics approaches, and computational analysis. Thus, the identification and validation of bioparts designed for specific genetic engineering objectives are also key steps within the design-build-test-learn (DBTL) framework of plant synthetic biology. In this project, we aim to identify novel plant bioparts, evaluate their functionality, and establish a biopart banking system to support their sharing and further reuse. To date, we have focused on the identification of candidate promoters by integrating transcriptome data with *in silico* motif analysis. To further enhance the precision and high-throughput identification and evaluation of bioparts, we try to incorporate ATAC-seq analysis for chromatin accessibility profiling and apply the biofoundry platform for automated, high-throughput functional screening. This integrated strategy is expected to enable more efficient development and utilization of robust bioparts, ultimately supporting scalable design in plant synthetic biology.

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Biochemical insights into a chitinase involved in the digestion of *Drosera binata*

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Keywords: enzymatic parameters, hydrolytic enzymes, recombinant protein, sundew

The genus *Drosera* L. (sundew) is among the largest groups of carnivorous plants, comprising over 250 tropical and temperate species. *Drosera binata* thrives in acidic, nutrient-poor soils and has evolved specialized leaves with structures known as "flytraps." These structures actively respond to prey contact, stimulating the production of hydrolytic enzymes that facilitate insect digestion. Among these enzymes, chitinases play a crucial role in breaking down the chitin exoskeleton of arthropods. This study aimed to characterize the biochemical properties of a specific chitinase, belonging to the class I chitinase family with catalytic and chitin-binding domains, involved in insect prey degradation. The open reading frame of the *D. binata* chitinase gene, excluding the signal peptide, was amplified via PCR, cloned into the pET-K2 expression vector without the thioredoxin fusion, and introduced into the *Escherichia coli* BL21-CodonPlus expression strain. Following the induction of protein expression, the recombinant chitinase (DbChitI-3) was purified using the Ni-NTA agarose column (Qiagen) and Econo-Pac 10DG Columns (Bio-Rad). Chitinolytic activity was measured fluorometrically using acid-swollen FITC-chitin as substrate. Enzyme activity was assessed across a broad range of pH and temperature, with maximum activity observed at pH 2.5-3.0 and temperatures between 15-30 °C. The enzyme effectively cleaved swollen FITC-chitin and glycol chitin but did not hydrolyze any shorter amino sugar substrates. These findings indicate that DbChitI-3 demonstrates endochitinase activity but does not exhibit β-N-acetylglucosaminidase, chitobiosidase, or short chitooligomer-specific endochitinase activity. The identified and characterized class I chitinase DbChitI-3 expands the known repertoire of chitinases in the genus *Drosera*. The highly acidic nature of digestive fluids in carnivorous plants is critical for prey decomposition and nutrient absorption. Consistent with this, the presented recombinant *D. binata* chitinase showed peak activity under strongly acidic conditions, similar to chitinases found in the stomachs or intestines of omnivorous animals. Further structural and molecular analyses will investigate the potential biotechnological applications of DbChitI-3 and its hydrolysis products.

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The contribution of VND family transcription factors to the diversity of plant secondary cell walls

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Keywords: plant cell walls, xylogenesis, VND transcription factors

Plant cells are surrounded by cell walls (CWs) composed of cellulose, various hemicelluloses, and lignin. CW composition and architecture vary significantly across plant species and tissues, providing specific properties. In conifer wood, two tracheid types, earlywood and latewood, exhibit distinct anatomical and biochemical features, mainly in hemicellulose and lignin structures[1]. To explore the genetic basis of these biochemical differences, we identified *Picea abies* VND (VASCULAR-RELATED NAC-DOMAIN) transcription factor homologs (*Pa*VNDs) and confirmed their role in CW deposition. Controlled growth experiments in trees, modelling the earlywood-to-latewood transition, revealed light-dependent *VND* expression, suggesting transcriptional regulation of xylogenesis. To examine *Pa*VND function in CW assembly, we assessed CW composition after transient overexpression in *N. benthamiana* leaves. Biochemical and gene expression analyses showed that each *Pa*VND differentially regulates CW formation. Moreover, Raman spectroscopy provided insights into CW assembly dynamics by visualizing local changes in

periclinal and anticlinal walls. Further experiments suggested that *PaVND* activity may be influenced by cellular redox homeostasis and protein interactions, adding another regulatory layer to CW formation. Expanding CW studies beyond dicot models, we attempted to develop a CW induction system in gymnosperm protoplasts, aiming to establish a novel model for xylogenesis studies and gain evolutionary insights into CW formation in conifers. To support comparative studies across species, we developed software enabling database handling and preparation of ready-to-use input files for phylogenetic analyses of multiple species. Our findings improve understanding of CW transcriptional control, paving the way for wood modification strategies and providing insights into *Pa*VND function in CW composition.

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Analysis of the possible function of class II TREHALOSE PHOSPHATE SYNTHASE (TPS) 5 and TPS6 genes by their overexpression in *Arabidopsis thaliana*

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Light-activated photosynthetic processes are crucial for carbon (C)-dependent plants. Therefore, a tightly regulated signaling network in needed to regulate C assimilation, storage and use for development and growth. One of the main signaling pathways involved in the control of C allocation in plants is trehalose-6-phosphate (T6P). Among the 11 *TREHALOSE PHOSPHATE SYNTHASE* genes in found in *A. thaliana*, only those grouped into Class I, i.e., *TPS1-4*, have members having the capacity to synthesize T6P, while the function of class II *TPS* genes remains poorly understood. In this context, transgenic *A. thaliana* plants overexpressing (OE) the class II *AtTPS5* and *AtTPS6* genes, which showed the most altered morphological phenotypes compared to WT plants, were selected for further study to gain a better comprehension of their role in plants, particularly regarding their relationship with parameters related to the regulation of C metabolism and growth. The results obtained with both OE plant lines have so far have indicated clear differences with WT plants and sometimes with each other in various development-related traits such as seed germination, early root development, rosette vegetative growth, flowering time and inflorescence size, sensitivity to increased levels of abscisic acid, trehalose and sucrose, ATPase activity in roots and leaves, and seed yield. A transcriptomic analysis of both OE lines is underway to detect the expression of key genes mostly involved in C and trehalose metabolism and its control, phytohormone signaling and regulation, membrane transport and consequent growth and development, among others, to provide a possible explanation of the results obtained and, thereby, a more precise knowledge of the physiological role in plants.

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Functional analysis of whirly protein in *Arabidopsis thaliana*

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Keywords: ubiquitination, promoter, stress treatment

WHIRLY(WHY) proteins regulate various physiological processes in plants, including chloroplast development, stress response, and gene expression. In *Arabidopsis thaliana*, WHY1, WHY2, and WHY3 maintain the structural integrity of chloroplast and mitochondrial genomes and facilitate coordinated activities between organelles and the nucleus. Preliminary data showed that AtWHIRLY proteins are degraded by the ubiquitin-proteome pathway (UPS), but the turnover of WHIRLIES and the possible involvement of WHIRLIES ubiquitination are unknown. To investigate the transcriptional regulation of WHIRLY genes, we used the PLACE bioinformatics tool and promoter-GUS (β-glucuronidase) reporter assays to identify and characterize cis-regulatory elements within the 1kb upstream region of WHIRLY genes. PLACE bioinformatic tool revealed putative regulatory motifs associated with various abiotic and hormonal stresses. Subsequently, we assessed these promoters' spatial and temporal activity patterns by analyzing GUS expression in transgenic Arabidopsis lines under different stress conditions. We elucidated the regulatory mechanisms underlying the dynamic expression of WHIRLY genes in

response to environmental cues. To elucidate WHIRLY protein turnover, we investigated the protein-protein interactions of WHIRLY proteins by Mass Spectrometry. Cell-free degradation assay confirmed the involvement of UPL1, UPL2 and KEG E3ligases in the degradation of WHY2 and WHY3 protein. The next step will be to identify the specific substrate targeted by the E3 ligases in the WHIRLY complex. In addition, studying the role of WHIRLY-E3 ligase interactions in different plant species may reveal unique mechanisms of protein stability and regulation, increasing the potential for agricultural applications such as crop improvement.

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How olive fruits make their oil: A transcriptomic and fatty acid profiling study

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Keywords: olive, fruit maturation, olive oil, fatty acids, RNA-seq, triacylglycerols

Olive oil is a rich source of health-promoting fatty acids (FAs), establishing olive trees as one of the most important crop species globally. Despite their significance, the transcriptional regulation of FA biosynthesis during olive drupe maturation remains poorly understood. In this study, we investigated olive oil biosynthesis and composition through an integrated transcriptomic and targeted FA analysis across four distinct developmental stages of drupes and the extracted oil from the emblematic cultivar 'Koroneiki'. Our findings revealed a significant increase in monounsaturated FAs (MUFAs) and polyunsaturated FAs (PUFAs) during fruit maturation, accompanied by a gradual decline in saturated FAs (SFAs). Transcriptomic analysis identified key marker genes involved in FA biosynthesis. Notably, a homolog of *β-ketoacyl-ACP synthase II* (*KAS II*) and two *fatty acid thioesterase A* (*FATA*) homologs were classified as potential markers for MUFA biosynthesis. Similarly, two *FATB* isoforms exhibited declining expression patterns consistent with the observed reduction in SFA content. Genes crucial for linoleic acid biosynthesis, such as *OeFAD2-5* and *OeFAD6*, showed progressively increasing expression during maturation, correlating with their corresponding metabolite levels. Additionally, the expression of two *FAD7* homologs was closely aligned with the accumulation trend of linolenic acid. Genes involved in the later steps of the Kennedy pathway, along with *oleosin-encoding* genes, also exhibited enhanced expression, supporting triacylglycerol (TAG) biosynthesis and accumulation within oil bodies. This study provides valuable knowledge about the molecular regulation of olive oil biosynthesis, offering insights for optimizing high-quality extra virgin olive oil production through the integration of applied research and precision agriculture.

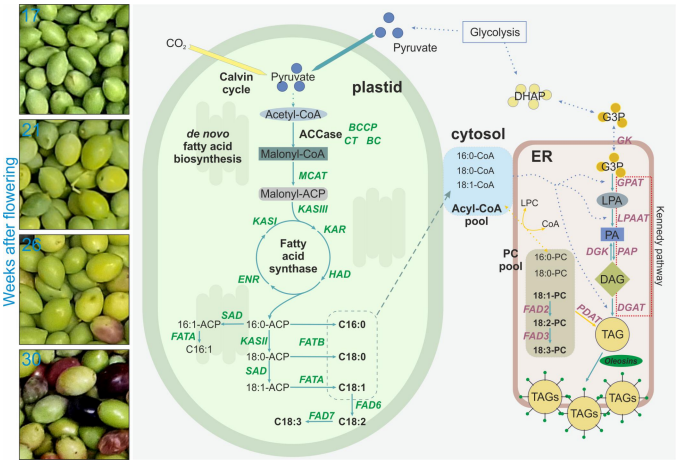

Figure 1: *Fatty Acid Metabolism and Oil Accumulation During 'Koroneiki' Olive Drupe Development.*

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Comparative analysis of immunogenicity and protection of Nipah virus vaccine candidates produced in *Nicotiana benthamiana*

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Keywords: Nipah virus, Nicotiana benthamiana, plant-produced vaccines

Nipah virus is a zoonotic pathogen that causes severe respiratory disease and encephalitis in humans, with a high mortality rate. Originating in Southeast Asia, NiV poses a significant public health threat due to sporadic out breaks and the absence of effective treatments, necessitating rapid vaccine development. In this study, we utilized *Nicotiana benthamiana* to produce NiV antigen proteins, specifically NiV-F and NiV-F+G trimers, using a transient expression system. We compared the immunogenicity and efficacy of these plant-derived antigens to those produced in CHO cells. We assessed antibody production and evaluated in vivo vaccine efficacy using a pseudovirus infection model in vaccinated mice. Our results demonstrated that the plant-produced NiV-F and NiV-F+G elicited stronger protective immune responses against NiV compared to their CHO derived counterparts. Additionally, we established a stable transgenic *N. benthamiana* line expressing NiV-F+G, facilitating rapid and scalable vaccine production. These findings underscore the potential of plant-based systems for efficient vaccine production, offering a cost-effective and convenient solution to address emerging viral threats.

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CELL BIOLOGY

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Auxin's role during endoparasitic nematodes feeding-sites formation

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Keywords: auxins, giant cells, syncytia

Plant-parasitic nematodes (PPNs), including cysts (CNs) and root-knot nematodes (RKNs), are known to cause serious agronomic losses worldwide, damaging a wide variety of crops. These two groups of PPNs induce, through a complex parasitism behavior, specialized feeding cells that become the only source of nutrients for nematode's development. RKNs develop giant cells (GCs) which are multinucleated cells with highly increased volume and a dense cytoplasm from vascular cells that undergo repeated mitosis with partial cytokinesis and DNA endoreduplication. Indeed, plant tissues around the GCs usually respond forming a pseudo-organ named gall that contains the GCs. CNs, on the other hand, form syncytia by selecting cambial or procambial cells that become the initial syncytial cells. The syncytium is then formed by the incorporation of neighboring root cells through local cell wall dissolution and shares some characteristics with GCs, such as endoreduplication and a dense cytosol. Despite the fact that the ontogenesis of syncytia and GCs is strikingly different an auxin maximum is crucial for the formation of both structures. In order to elucidate the molecular divergences of these structures, we have investigated the auxin transduction pathways during the CNs interaction, focusing on several genes that are key during gall and lateral root development.

The promoter *pGATA23* and several deletions of *pmiR390a* were active in both galls and syncytia, whilst *AHP6* and/or putative up-stream regulators as *ARF5/7/19* were only active in galls and not in syncytia. Nevertheless, none of these genes seemed to display a key role during CNs establishment. The analysis of those promoters active only in galls *(AHP6, LBD16*) revealed the presence of only canonical AuxRe elements in their proximal promoter regions, whereas the analyzed promoters active in syncytia such as *pmiR390a* and *pGATA23* carry AuxRe overlapping core cis-elements for other transcription factor families (i.e., bHLH, bZIP). Furthemore, *in silico* transcriptomic analysis showed, despite the high number of IAA induced genes present in both galls and syncytia compared to uninfected roots, a few common upregulated IAA responsive genes in both types of feeding sites. These results suggest that in both interactions operate complex and partially divergent regulatory pathways driven by auxins.

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P-48

RGII dimerization defects uncover a new pathway for cell adhesion in *Arabidopsis*

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Keywords: adhesion, cell wall integrity, brassinosteroid

Cell-cell adhesion is a fundamental feature for the maintenance of multicellular integrity. In plants, the cell wall is responsible for maintaining neighboring cell adhesion. Here in this study, we discover the role of Rhamnogalacturonan II (RGII), a component in the cell wall, as a key regulator of plant cell adhesion and integrity. We demonstrate that cell adhesion defects are present in RGII dimerization deficient mutant *mur1*, which occurs via a different mechanism than the previously identified pectin-deficient mutants. The adhesion phenotype caused by the RGII dimerization problem is subsequently demonstrated to be mostly caused by the misregulation of the brassinosteroid pathway and the impaired cell layer growth coordination. Finally, by characterizing the chemical and mechanical properties of the cell wall, we demonstrate that the RGII dimerization defect renders the cell wall mechanically weaker. We conclude that RGII dimerization defects, directly and indirectly, affect cell wall mechanics through cell wall remodeling, brassinosteroid signaling, and cell layer growth coordination. Future work will bring insights into the brassinosteroid signaling network in coordinating the adhesion and growth mechanics in Arabidopsis.

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P-49

Functional characterization of NADPH-cytochrome P450 reductase driven from tomato

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Keywords: tomato, NADPH-cytochrome P450 reductase, heterologous expression

NADPH-cytochrome P450 reductase (CPR) is a key enzyme that transfers electrons to cytochrome P450. Plants cytochrome P450s play an important role in various metabolisms, including biosynthesis, mechanism of hormones and defense mechanisms. In tomatoes (*Solanum lycopersicum*), two putative *CPR* genes, *SlCPR1*

and *SlCPR2*, were identified. *SlCPR1* was constitutively expressed, whereas *SlCPR2* expression was induced significantly by jasmonic acid treatment. No notable changes in expression were detected following salicylic acid treatment or under drought stress. To characterize the enzymatic properties of SlCPRs, their cDNAs were expressed without any amino acid modification. The purified SlCPR enzymes were tested with various protein and chemical substrates. SlCPR2 showed higher activity than SlCPR1 for all substrates tested. Both SlCPRs exhibited strong activity across a pH range of 6.0 to 9.0 when using MTT as a substrate, with the highest activity observed at pH 8.0. This study opens possibilities for CPR control, biocatalyst development, and exploring oxidase enzyme functions. Furthermore, transgenic tomatoes have been developed in which each *CPR* gene has been either knocked out or overexpressed. This approach is expected to provide insights into the mechanistic relationship between individual CPR isoforms and P450 enzymes in tomato.

P-50

Centromere dynamics in mitotic and meiotic cells of common barley as revealed by the construction of CENH3-dsRed fluorescent reporter lines

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Centromeres are chromosomal regions responsible for chromosome movement throughout mitotic and meiotic cell division. During prophase I of meiosis, when the chromatin is enclosed in the nuclear envelope the spatial arrangement of centromeres plays a crucial role in chromatin organisation, homologous chromosome recognition and recombination. A characteristic centromere dynamics in the close proximity of the nuclear envelope is observed almost universally in sexually reproducing organisms. Programmed centromere-centromere associations and abrupt changes in spatial arrangements with respect to the nuclear envelope accompany chromosome pairing and meiotic recombination. Gamete fertility and genetic diversity rely on these processes therefore their effectiveness is crucial for crop production under the changing climate. Despite the fundamental role of centromeres in cell division, their organisation and movement in the meiocytes of large genome cereals is still enigmatic to date. Centromere activity is determined by the presence of the centromeric histone H3 (CENH3) protein within the nucleosomes, and some plants, such as barley or wheat, produce two protein variants: aCENH3 and bCENH3. The aim of the present study is to capture *in vivo* meiotic centromere dynamics in barley (*Hordeum vulgare*) in order to monitor prophase movement in real time. We aimed to produce CENH3 fluorescent reporter lines that allow a clear and specific labelling of centromeric regions in both somatic and meiotic cells. By using the barley bCENH3 cDNA sequence we created a transgenic line expressing the bCENH3 coding sequence fused to Discosoma Red (dsRed) fluorescent protein, driven by a 35S promoter. High-resolution confocal microscopy analysis of mitotic cells detected a stable centromere-specific fluorescent signal within the cell nuclei, revealing the number of centromeres and their localisation with respect to the nuclear envelope. Our study demonstrates that the introgression of the p35S-bCENH3-dsRed reporter cassette in barley plants serves as a reliable methodology for determining key cellular dynamics in the mitotic cells of barley. Our study allows further analysis of meiotic cells to understand the contribution of centromere movement to correct chromosome recognition and pairing, a prerequisite for fertility and genetic diversity in crops.

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P-51

Computational modelling of auxin, cytokinin, and salicylic acid binding by tomato Phi class glutathione transferases

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Keywords: glutathione transferases, AlphaFold protein modelling, molecular docking, protein-ligand interactions, molecular dynamics simulations

Glutathione transferases (GSTs, EC 2.5.1.18) are a diverse enzyme family involved in various cellular functions and regulated by multiple stimuli, including phytohormones. Interestingly, some GSTs, particularly from the Phi class (GSTFs), have been reported to directly bind small phytohormone molecules such as auxin, cytokinin, and salicylic acid–suggesting a potential role in modulating hormone levels. However, the structural basis of these interactions remains unclear. In *Arabidopsis thaliana*, the AtGSTF2 is known to possess distinct non-catalytic ligand-binding sites (L sites), raising questions about similar functions in orthologous proteins from other species. This study investigates the tomato (*Solanum lycopersicum*) GSTFs (SlGSTFs) to explore their capacity for phytohormone binding via these non-catalytic sites. Using various computational approaches, such as the widely recognized AlphaFold protein modelling, molecular docking, short molecular dynamics simulations, and simple root mean square deviation (RMSD) analysis to describe protein-ligand interactions, we present the first evidence supporting phytohormone binding in SlGSTFs. Notably, SlGSTF4 and SlGSTF5 exhibit strong phytohormone-binding potential, suggesting an expanded functional repertoire for plant GSTs beyond their traditional enzymatic roles.

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P-52

Korean red ginseng main component, Rb1, attenuated particulate matter-induced senescence in skin keratinocytes

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Keywords: Rb1; particulate matter 2.5, human keratinocyte, senescence

Recently some studies has reported that ambient air particulate matter (PM) induces senescence in human skin cells. In this study, we investigated whether Korean red ginseng may attenuate fine particulate matter ($PM_{2.5}$)-induced senescence of skin keratinocytes. The human keratinocyte cell lines HaCaT and normal human epidermal keratinocytes (NHEK) were treated with $PM_{2.5}$ at 50 μg/ml. $PM_{2.5}$-treated cells showed characteristics of cellular senescence, including an enlarged and flattened cell shape and irregular size, and decreased colony-forming ability, however, Korean red ginseng main component, Rb1, suppressed these characteristics of cellular senescence in both cell types. And $PM_{2.5}$-treated cells exhibited β-galactosidase activity in the cytosol, a characteristic of cellular senescence, as evidenced by higher levels of green signal in $PM_{2.5}$-treated cells than in untreated cells, however, Rb1 suppressed the β-galactosidase activity in both cell types. Chromatin in senescent cells undergoes large-scale rearrangements, forming dense nuclear domains called senescence-associated heterochromatin foci (SAHF). $PM_{2.5}$-treated cells displayed significantly more SAHF in the nuclei than the controls, however, Rb1 decreased the number of SAHF. Moreover, the expression of $p16^{INK4A}$, a CDK inhibitor and senescence inducer, was strongly increased in $PM_{2.5}$-treated cells compared to the corresponding untreated cells, however, Rb1 decreased the expression of $p16^{INK4A}$. These results suggest that Rb1 have the protective effects against $PM_{2.5}$-induced senescence of skin keratinocytes (RS-2023-00270936).

P-53

Deciphering protein interactome of *Solanum lycopersicum* through yeast two-hybrid high throughput screening

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Keywords: protein interactions, yeast two-hybrid, cell biology

Understanding protein interaction networks provides crucial insights into the molecular mechanisms of signal transduction, stress responses, and developmental processes. When applied to plant science, the analysis of these networks can pave the way for yield improvement, enhancement of traits relevant to agronomically important crops and vegetables, and a better understanding of the host-pathogen interactions, with the final aim of effective pest management. Yeast two-hybrid (Y2H) protein interaction screening and related techniques have proven instrumental in identifying new protein partners for proteins, DNA, RNA or small molecules.

At Hybrigenics, we employ yeast two-hybrid technology and a domain-based screening strategy to ensure reproducible and exhaustive Y2H results. Our custom-made, highly complex fragment libraries - of more than 10 million independent fragments in yeast - are screened to saturation using an optimized mating procedure that allows us to test, on average, 83 million interactions per screen, corresponding to an 8-fold coverage of the library. As a consequence, multiple, independent fragments are isolated for each interactant, enabling the immediate delineation of a minimal interacting domain and the computation of a confidence score.

To date, we have created over 50 cDNA libraries for protein interaction screening from 23 plant species (e.g. Arabidopsis thaliana, Nicotiana benthamiana, Solanum lycopersicum, Oryza sativa) which are available on a fee-for-service basis. Preparation of additional cDNA libraries is currently in progress. Furthermore, we have developed several methods to validate and characterize interactions identified using Y2H screens or other protein interaction techniques through the pairwise testing of the interaction between two selected proteins ("1by1 assays").

Here, we present screening results from one of our highly complex tomato libraries (vegetative meristems, reproductive meristems, P3-P5 leaf primordia + stems, and floral buds) using MEK1 as the bait protein. These results demonstrate the power of the yeast screening technology to elucidate interaction pathways in plants and in the Solanaceae family and encourage further explorations of plant protein interactome.

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P-54

Toolbox for measuring *in vitro* and *in planta* cAMP production

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Keywords: cAMP, LC-MS, sensors, microfluidics

In animals, cyclic nucleotide monophosphates (cNMPs) are classical and well-established second messengers. Cyclic adenosine monophosphate (cAMP) is produced by the action of adenylyl cyclases (ACs) in response to various external stimuli like hormones, neurotransmitters, and environmental signals. Although extensively studied in animal systems, the production and functional roles of cAMP in plants have only gained significant attention recently. ACs exist as moonlighting proteins in plants, possessing additional functions not obviously related to cAMP production making their identification and characterization challenging. Notably, the TIR1-type auxin receptors have recently been discovered to have AC activity, which is essential for auxin signaling (Qi *et al.*, 2022; Chen *et al.*, 2025). To better study cAMP in plants, we present an integrated toolkit for measuring cAMP, combining LC-MS-based metabolic approach and transgenic biosensor plant lines. We established methods for *in vitro* measurements of AC activities of purified proteins and *in planta* measurements of cAMP content in response to different signals. Additionally, the biosensors from the animal field, cAMPr (Hackley *et al.*, 2018) and cAMPinG1 (Yokoyama *et* al., 2024), were adapted for plant systems to enable real-time monitoring of cAMP dynamics. Microfluidics-based approaches were employed to apply hormone, biotic, and abiotic stress treatment and monitor cAMP production with high spatial and temporal resolution demonstrating an applicability of this approach. Thus, this integrated strategy promises to greatly advance the studies on role of cAMP in plants.

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P-55

Regulatory role of spermidine during leaf senescence via eIF5A (in)dependent manner

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Keywords: hypusine, spermidine, eIF5A

Senescence is highly controlled and active process requiring global metabolic reprogramming. It aims at organizing disintegration and remobilization of valuable resources. Senescence is a fundamental aspect of plant development, necessary to optimize resource allocation and promote phenotypic plasticity to adapt to the environment under restricted conditions.

Polyamines (PAs), such as putrescine, spermidine (SPD), and spermine, are essential polycationic biogenic amines found in all eukaryotes and are crucial for cell survival. During leaf senescence, the expression of S-adenosylmethionine decarboxylase and spermidine synthase gene families undergoes significant changes, indicating their pivotal role in SPD metabolism and may condition metabolic reprogramming. The research carried out by our group, allowed us to develop a model of PA action on Dark-Induced Leaf Senescence (DILS) program, indicating that the direction of SPD metabolism, towards its anabolism or catabolism, plays a central role in metabolic reprogramming, which introduces or not senescing leaves into the programmed organ death.

Spermidine plays also a key role in eukaryotes by providing the 4-aminobutyl group necessary for the posttranslational modification of lysine in eukaryotic initiation translation factor 5A (eIF5A) into the unusual amino acid hypusine. This modification involves two sequential enzymatic steps. The first step which provides the 4-aminobutyl group destined for the lysine of eIF5A, forming an intermediate deoxyhypusine is catalyzed by deoxyhypusine synthase. Thus, hypusine synthesis defines an absolute requirement for the SPD in eukaryotes, showing essential functions of SPD in plant responses to stress. The intermediate deoxyhypusine is further converted to hypusine by deoxyhypusine hydroxylase, thereby activating eIF5A. While eIF5A is well-known for its role in translating proline-rich repeat proteins, its biological function has been recently elucidated in mammals but remains poorly understood at a functional level in plants. The expression patterns of *eIF5A* isoforms and the genes responsible for hypusination vary between induced and developmental leaf senescence (DLS). During DILS we observed an enhanced expression of *HveIF5A1* and *HveIF5A3*, while *HveIF5A2* was notably inhibited. In contrast, during DLS, *HveIF5A2* and *HveIF5A3* exhibit opposite expression dynamics compared to DILS. Additionally, *HvDHS* shows an upregulation trend in both DILS and DLS, whereas *HvDOHH* expression is significantly elevated only during DILS.

Presenting preliminary results on this topic, we propose that SPD may act as a biological switch regulating different types of senescence differently through both eIF5A-dependent and independent pathways.

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P-56

Eckol attenuates fine particulate matter-induced cellular senescence by regulating antioxidant enzymes and matrix metalloproteinases

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Keywords: Eckol, particulate matter, antioxidant enzymes, matrix metalloproteinases

Airborne fine particulate matter ($PM_{2.5}$) is a major environmental pollutant that accelerates skin aging through oxidative stress and matrix metalloproteinase (MMP) activation. This study investigates the protective effects of eckol, a phlorotannin isolated from brown algae, against $PM_{2.5}$-induced senescence in HaCaT keratinocytes. Eckol treatment increased the expression of antioxidant enzymes, superoxide dismutase (SOD) and

catalase, whereas $PM_{2.5}$ exposure led to a significant reduction in these enzymes. $PM_{2.5}$ exposure increased the expression and activation of MMP-1, MMP-2, and MMP-9 in a time-dependent manner, with significant induction observed at 36 to 48 hours. Eckol suppressed the $PM_{2.5}$-induced upregulation of MMPs at both the mRNA and protein levels. $PM_{2.5}$ activated MEK/ERK and SEK/JNK pathways, leading to increased phosphorylation of c-Fos and c-Jun, key components of the activator protein 1 (AP-1) transcription factor. Chromatin immunoprecipitation assays confirmed enhanced AP-1 binding to the MMP-1 promoter upon $PM_{2.5}$ exposure, which was significantly mitigated by eckol restored $PM_{2.5}$-induced depletion of SOD and catalase, reducing intracellular reactive oxygen species accumulation. siRNA-mediated knockdown of SOD and catalase abolished eckol's inhibitory effects on MMP expression. These findings highlight the potential of eckol as a protective agent against $PM_{2.5}$-induced skin aging through the regulation of oxidative stress and MMP expression, suggesting its therapeutic application in skin care and anti-aging formulations (RS-2023-00270936).

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P-57

Adenylate cyclase activity of 14-3-3 proteins in plants

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Keywords: 14-3-3 proteins, adenylate cyclase activity, cyclic AMP

Arabidopsis 14-3-3 proteins are a family of regulatory proteins known to interact with phosphorylated serine/threonine motifs on target proteins, thereby modulating their enzymatic activity, subcellular localization, or stability. They can be found in all eukaryotes and are involved in diverse signaling pathways, including hormone and stress responses in plants. Here, we identify that certain plant 14-3-3 isoforms exhibit adenylate cyclase (AC) activity, enabling the direct conversion of ATP to cyclic AMP (cAMP). This potential enzymatic function broadens our understanding of 14-3-3 proteins, suggesting that they may contribute to second messenger signaling rather than acting solely as scaffolds or adaptor proteins.

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CROP ADAPTATION TO CLIMATE CHANGE

P-58

Chickpea for sustainable agriculture under a changing climate in Hungary: Effects of sowing date on yield and seed quality under supplemental irrigation

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Keywords: crop rotation, drought, legumes

Legume cultivation is gaining more attention in Europe, including Hungary. However, chickpea cultivation is still underestimated and less is known about the adaptability of chickpea to crop rotations with cereals in Hungary. In particular, the possibility of cultivating chickpea after wheat harvest, leading to a sustainable, all-year-long land exploitation, in addition to the necessity of supplemental irrigation under the current climatic conditions in Hungary are yet to be evaluated. We conducted an experiment in the experimental fields of the Faculty of Agricultural and Food Sciences and Environmental Management of the University of Debrecen in 2024 aiming to answer these questions. Three chickpea varieties (Amorgos, Elmo, and Orion) were cultivated in spring (conventional sowing date) and in the summer (late sowing date, after wheat harvest) under two irrigation schemes: supplemental irrigation (where water availability was monitored, and irrigation was applied as necessary) and rainfed conditions. The experiment was laid out in a randomized complete block design with four replicates. Neither supplemental irrigation, nor the late sowing date did influence the seed yield of the studied varieties. However, significant seed yield differences were recorded among varieties, where "Orion" had the highest, and "Elmo" the lowest seed yield, regardless of irrigation scheme or sowing date. Supplemental irrigation did not result in measurable differences in the seed's dietary fiber concentration of any variety. However, the late sowing date resulted in significant increase in the dietary fiber concentration in the seeds of both "Amorgos" and "Orion" varieties under supplemental irrigation conditions, whereas the increase was only significant in "Elmo" variety under rainfed conditions, reflecting variety-dependent response. "Elmo" seeds had significantly higher dietary fiber concentration as compared to the other varieties under both irrigation schemes and on both sowing dates. It

could be concluded that field sustainable utilization, through a wheat-chickpea crop rotation across the cropping year, seems feasible and promising. However, selecting suitable chickpea varieties seems vital. Our future research will focus on continuing this research in the long term to get reliable conclusions, in addition to expanding the experimental tests to include the evaluation of possible reductions in chemical nitrogen demands by wheat plants in this crop rotation and, hence, the possible economic and environmental gains.

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P-59

Pre-hardening induced by blue or far-red light supplementation in leaves of the Rht12 wheat dwarfing line: Effects on metabolism, freezing tolerance, and gibberellin levels

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Keywords: light-regulation, gibberellin, freezing tolerance

In addition to drought and heatwaves, climate change presents new challenges for cereals in terms of their freezing tolerance. Milder winters and increased temperature fluctuations are expected to reduce the effectiveness of cold-induced freezing tolerance (cold acclimation), while the frequency of sudden, extreme cold events is predicted to rise. Under such conditions, unacclimated or de-acclimated plants will likely suffer significant frost damage, leading to yield losses. As a result, short cold (non-freezing) exposures–referred to as pre-hardening (lasting approximately one week)–and other environmental factors, such as light quality, will play a more prominent role in determining the freezing tolerance of young winter cereals than in the past. Contrastingly to later stages of cold acclimation (after several weeks), gibberellin (GA) levels fluctuate dynamically during pre-hardening, underlining their importance in the process. The introduction of Reduced height (*Rht*) genes in wheat breeding has revolutionised wheat production; however, these genes can compromise the plant's ability to withstand freezing temperatures. Currently, only a few *Rht* alleles are used in cultivated wheat varieties, emphasizing the need to explore how other variants respond to environmental stress. Most mutant *Rht* alleles exert a dominant effect on GA signaling or availability. In winter cereals, supplementing white light with far-red light (W+FR) at 15°C can partially induce pre-hardening. In contrast, the effects of blue light enrichment (W+B) under similar conditions remain less understood. This study examines how 10 days of exposure to W+FR and W+B light treatments at 15°C influence young winter wheat leaves. It compares a tall, wild-type genotype (*rht12*) with a dwarf, GA-deficient near-isogenic line (*Rht12*), both derived from the Maris Huntsman variety. The main objective was to determine how different light conditions together with GA deficiency affect leaf freezing tolerance during light-quality induced pre-hardening. To this end, several parameters were assessed, including frost damage, bioactive GA content, metabolic changes, and relevant gene expression of leaves. The results indicate that *Rht* alleles influence the inherent freezing resistance of wheat leaves by modulating GA levels. Both W+FR and W+B light treatments helped mitigate the freezing-sensitive traits of *Rht12* leaves, suggesting the presence of compensatory mechanisms. Differences in GA metabolism between the two genotypes under varying light conditions were associated with contrasting levels of freezing tolerance. These findings suggest that modifying light environments could be an effective strategy for improving freezing tolerance in dwarf wheat varieties with limited GA synthesis.

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P-60

The influence of high temperature and elevated CO_2 concentration on potato growth and yield

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Keywords: potato, carbon dioxide, temperature, photosynthesis, yield

Global climate change accompanied by continuous increases in temperature and atmospheric carbon dioxide (CO_2) concentration affect the growth and yield of crops. Accordingly, the present study was conducted to investigate effects of elevated temperature and/or CO_2 concentrations on the growth, photosynthesis and yield of potato crops using Korean Soil-Plant-Atmosphere-Research (KSPAR) chambers and Temperature Gradient Field Chambers(TGFC). Potato plants were exposed to four different treatment conditions: ambient weather (aCaT), elevated temperature (aCeT), elevated CO_2 concentration (eCaT), and concurrently elevated CO_2 and temperature (eCeT). Under aCeT conditions, the temperature exceeded the optimal growth temperature range towards the late-growth phase, thereby reducing stomatal conductance and the canopy net photosynthetic rate, which subsequently reduced biomass and tuber yield. In addition, the flowering initiation time of potatoes was accelerated about 5~9 days under the elevated temperature conditions at the SPAR and TGFC. Stomatal conductance and chlorophyll concentration were lower under eCaT conditions than under aCaT conditions, whereas late-growth phase biomass and tuber yield were greater. Compared with other conditions, the eCeT treatment yielded a distinct increase in growth and development as well as in the canopy net photosynthetic rate between tuber initiation and bulking. Consequently, biomass and canopy net photosynthesis increased, and this rise in yield may be attributed to increased tuber size rather than to increased tuber number. Elevated CO_2 concentrations reduced chlorophyll, magnesium, and phosphorus concentrations, whereas reduced nitrogen concentrations increased the C:N ratio. Taken together, these results indicate that future climate conditions will likely change the nutrient concentration and quality of crops. In conclusion, the present study reveals that the concurrent and appropriate elevation of CO_2 and temperature could promote the balanced development of source and sink organs and subsequently have positive effects on potato productivity and quality, even though elevated temperature may negatively influence the growth and yield of potato crops, especially towards the late-growth phase.

This work was supported by a grant from the Agenda project (No. PJ01357402) of the Rural Development Administration, Republic of Korea.

P-61

How does the transcriptomic profile of barley change when grown with the addition of inorganic carbon to the roots?

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Keywords: carbon metabolism, *Hordeum vulgare*, transcriptomics

Plants primarily absorb carbon dioxide through their leaves via photosynthesis, utilizing stomata to regulate gas exchange. However, research has revealed that roots can also uptake inorganic carbon, challenging the traditional understanding of plant carbon acquisition. This root-based carbon uptake can occur through specialized transporters in root cells or diffusion of CO_2. Studying the root-based uptake of inorganic carbon is crucial, as it will provide a more comprehensive understanding of plant carbon dynamics and their role in global carbon cycling.

In our study, we conducted a transcriptomic analysis of plants grown in a hydroponic system with 2 mM NaHCO3, ¼ Hoagland, and pH 5.6 as a growth-promoting treatment. We utilized leaves and roots from control and treated plants in triplicates. The objective of this analysis was to elucidate which pathways, transporters, channels, and genes were potentially associated with the uptake and translocation of inorganic carbon.

The primary results of our analysis indicate that the leaf tissue exhibited the most significant transcriptional changes when exposed to the treatment. Among the common genes upregulated in both leaves and roots, we identified various zinc transporters, nucleobase cation symporters, protein radialis-like proteins, nicotianamine

transferases, myoinositol 3 phosphate synthase, and pyrophosphate fructose 6 phosphate 1.

In the enrichment analysis, upregulated GO (gene ontology) terms for the leaves included cation transport, metal ion transmembrane transporter activity, and inorganic cation transmembrane transporter activity. For the roots, upregulated terms encompassed tricarboxylic acid metabolic process, polysaccharide metabolic process, anatomical structure development, and nicotianamine metabolic process. Upregulated KEGG (Kyoto Encyclopedia of Genes and Genomes) terms for the roots included glycolysis/gluconeogenesis, pentose and glucoronate interconversions, pyruvate metabolism, inositol phosphate metabolism, nitrogen metabolism, and alpha-linolenic acid metabolism.

In conclusion, our findings suggest that barley exhibits a distinct response compared to previous results obtained in Arabidopsis. Barley grown under growth-promoting conditions demonstrated activation of anabolic metabolism principally in the roots. This implies that the plants are utilizing carbon for growing while simultaneously storing energy despite the presence of stress pathways like the nicotianamine metabolic process.

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P-62

Cis-natural antisense transcript *Dof2NAT* promotes early heading and drought tolerance by increasing *Dof2* expression in rice

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Keywords: natural antisense transcript, early heading, drought tolerance, rice

Natural antisense transcripts (NATs) are transcribed in the direction opposite of their target mRNAs and act as key regulators of diverse biological processes in animals and plants. However, despite their widespread presence in plants, their functional roles remain largely unexplored, especially in heading date regulation and abiotic stress responses in rice. In this study, we investigate the functional role of the *OsDof2NAT/OsDof2* module in regulating heading date and drought tolerance in rice. The expression of *OsDof2NAT* and *OsDof2* is regulated by the circadian rhythm and induced under drought stress. *OsDof2NAT* enhances *OsDof2* expression by recruiting OsWDR5a, a component of the MLL complex, which promotes H3K4me3 deposition, whereas its knockdown reduces both *OsDof2* expression and H3K4me3 levels. Furthermore, overexpression of *OsDof2NAT* or *OsDof2* improves drought tolerance and promotes early heading by activating *Ehd1* and *Hd3a*. Our findings elucidate the molecular mechanism by which *OsDof2NAT* regulates *OsDof2* expression through histone modifications, underscoring the functional significance of lncRNAs in plant development and stress responses.

P-63

Increased expression of AtSPQ protein improves drought tolerance in *Brassica napus*

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Keywords: rapeseed, drought, reactive oxygen species

Brassica napus (rapeseed) is an important oilseed crop cultivated globally for edible oil and biofuel production, but its productivity is severely impacted by drought stress. Drought restricts leaf expansion, induces stomatal closure, and disrupts key physiological processes such as photosynthesis, respiration, and phytohormonal balance. Additionally, it leads to excessive accumulation of reactive oxygen species (ROS), which trigger antioxidant defense responses, phytohormone synthesis, and osmotic adjustments through organic solutes. These adaptive mechanisms help maintain cellular homeostasis and sustain photosynthetic activity, contributing to improved drought tolerance in plants. Previously, overexpression of the Small Paraquat resistant (SPQ) gene from *Arabidopsis thaliana* and *Lepidium crassifolium* was shown to improve drought tolerance in Arabidopsis by increasing viability, reducing oxidative damage, maintaining photosynthetic activity and increasing resistance to paraquat. In this study, Rapeseed, a close relative of Arabidopsis belonging to the same family and exhibiting sequence similarity, was transformed with *AtSPQ* using an optimized hypocotyl transformation method. Several parameters like physiological, biochemical and stress-induced gene expression of the successfully transformed lines were analysed. Preliminary results indicate that the AtSPQ-overexpressing lines exhibit enhanced viability,

suggesting that the *SPQ* gene may play an important role in improving overall plant health under stressed conditions. These findings have potential applications in drought mitigation and crop improvement strategies. Further studies are underway to investigate the underlying mechanisms in more detail.

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P-64

Transcriptome analysis of various sweet potato (*Ipomoea batatas*) cultivars in Taiwan using next-generation sequencing

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Keywords: sweet potato, next-generation sequencing, transcriptome analysis, gene function classification

With the ongoing challenges posed by global climate change and population growth, improving the yield, quality, stress resistance, and adaptability of sweet potatoes has become an urgent priority in agricultural research. In Taiwan, the breeding objectives for sweet potatoes focus on enhancing broad adaptability, high storage root yield, excellent quality, and the enrichment of functional compounds. These efforts aim to boost farmers' income and meet the growing public demand for health-promoting foods. Given that sweet potato germplasm in Taiwan is primarily preserved through vegetative propagation, resulting in limited genetic diversity, transcriptome-based approaches offer a promising avenue for efficiently identifying molecular traits and accelerating selection in breeding programs. This study utilized three major sweet potato cultivars, provided by the Chiayi Agricultural Experiment Branch of the Taiwan Agricultural Research Institute, as experimental materials: Tainung No. 57 (yellow skin and flesh, known for high yield and good eating quality), Tainung No. 66 (red skin and flesh, rich in carotenoids), and Tainung No. 73 (purple skin and flesh, rich in anthocyanins). A standardized RNA extraction protocol was developed for sweet potato storage root flesh, followed by next-generation sequencing. Differentially expressed genes among cultivars were analyzed using volcano plots to identify significantly upregulated and downregulated genes. Venn diagrams were employed to compare the numbers of shared and unique upregulated or downregulated genes among the different cultivars. Pearson correlation coefficient heatmaps were used to assess transcriptomic similarities and differences between cultivars. Gene function classification was performed based on known databases, with a functional categorization of metabolic pathways. Through these comprehensive analyses, this study aims to address the current lack of a complete transcriptomic database for Taiwanese sweet potatoes. The goal is to provide valuable physiological and molecular insights for sweet potato breeders, thereby advancing precision breeding in Taiwan while significantly reducing labor and cultivation space requirements.

P-65

A tuneable molecular approach to guarantee light-induced flowering for future climates

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Plants use the 24-hour light cycle to sense the passing of the seasons, which is needed for determining optimal flowering and seed dispersal times – a mechanism known as photoperiodism. Winter varieties of cereals like wheat and barley use temperature alongside photoperiod to regulate their flowering times, requiring a prolonged period of cold exposure in the winter months to become competent to flower in the spring. This process is known as vernalisation and it is threatened by climate change. Winters are projected to become warmer and shorter, such that future winters might not be sufficient to satisfy the vernalisation requirements of these crops, preventing their flowering. Warmer winters could also drive earlier flowering, which is equally as threatening to crop yields, as occasional winter frost can fatally damage the reproductive structures of the plant. Studying how light and temperature converge to regulate flowering times in winter cereals is therefore important for the future of agriculture and plant breeding in the face of our current climate crisis. In northern and central Europe, winter

wheat varieties are preferable to spring varieties (which have no vernalisation requirement), as winter varieties have better cold and frost tolerance, higher yields and excellent grain quality favourable for the bread industry. My PhD project aims to develop an innovative approach to ensure that winter wheat can flower based solely on changes in photoperiod and regardless of winter temperatures.

In order to achieve this, I will attempt to manipulate the expression levels of a recently discovered flowering regulator in wheat called ODDSOC2-2 (OS2-2). Natural allelic variation at the OS2-2 locus in winter wheat was observed to correlate with early flowering phenotypes.

Furthermore, the expression of OS2-2 was found to be regulated by both photoperiod and temperature, with the OS2-2 locus being a target of the vernalisation regulator VERNALIZATION 1 (VRN1). The short-day photoperiods of autumn promote the expression of OS2-2, whereas the cold temperatures of winter and long day photoperiods of spring repress OS2-2 expression. I will use a two-step approach to address my goals. First, I will investigate the molecular and epigenetic mechanisms underlying the regulation of OS2-2 expression to identify the molecular players involved in the temperature-dependent regulation of the gene. Second, I aim to identify small biomolecules that can target and inhibit this temperature-dependent regulation, allowing OS2-2 to be regulated solely by changes in photoperiod. Ultimately, we wish to develop an epigenetic drug which can be applied to the wheat crop in the case of a winter which is insufficiently cold and/or long, blocking the temperature-dependent regulation of flowering time and enabling proper flowering based only on light.

P-66

Foliar application of brassinosteroids: Impact on harvest and post-harvest quality of 'flame seedless' grapevine in open field and covered systems

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Keywords: Gene expression, plastic cover, antioxidant, cold storage, *Vitis vinifera* L.

Early grape maturation is a crucial trait for Egypt's grape export industry to meet international market demands in a timely manner. In a two-year study, a plastic covering technique was applied to 'Flame' seedless grapes, along with exogenous brassinosteroid (BR) treatments, to evaluate their effects on fruit quality at harvest and during storage.

The findings revealed that grapes grown under plastic covering exhibited accelerated maturation, with an average harvest date two weeks earlier than that of uncovered vines, regardless of BR treatment. However, this early maturation was associated with a reduction in anthocyanin content. Notably, BR application partially mitigated this deficiency while simultaneously improving fruit quality attributes and storage stability.

Additionally, a dose-dependent upregulation of key anthocyanin biosynthesis genes–CHS, F3H, and UFGT–was observed in response to BR treatment, correlating with a significant increase in anthocyanin accumulation.

Overall, our study suggests that combining plastic covering with an optimized BR concentration (0.8 ppm) is an effective strategy for advancing grape harvest timing while maintaining postharvest quality during cold storage and shelf life.

P-67

Biometrical and biochemical response to thermal stress in tomato plants grown under LED lighting

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Keywords: LED lighting, tomato, temperature

Temperature is an environmental factor affecting growth and development processes in seedlings. The climate change, including a shift in temperature patterns, causes metabolic perturbations and biometrical alterations in seedlings. This study explores the potential benefits of using light-emitting diodes (LED) technology to counteract and/or prevent these negative effects when tomato seedlings were grown under temperature stress conditions. Compared to conventional lighting, LED lighting allows to evaluate how a single monochromatic wavelength affects the responses in plants' resilience to temperature stress. Using LED technology, in addition to light quality, it is possible to control also its quantity. In this work, the growth of tomato seedlings of two Apulian varieties (Regina and Mola), grown under three specific monochromatic LEDs (blue, red and white light) at different light intensities (100 and 200 µmol m^{-2} s^{-1} of Photosynthetic Photon Flux Density) and temperatures (20 °C, 25 °C and 30 °C) was considered. The analyses were carried out at the early growth stages of the seedlings, including sowing and germinability, seedling morphology, carbohydrate content and stress indicators. Depending on the different employed LED lighting, the biochemical parameters analysed as well as the biometrical characteristics were differently affected. Particularly, the seedlings grown under blue light showed a growth inhibition and the presence of an oxidative state, although a potentiate defence response counteract it; no differences were observed increasing the light intensity and the obtained patterns were similar for the two considered tomato varieties. In presence of red light, the tomato seedlings show similar trends in the biometrical and biochemical characteristics compared to those observed under white light. In general, Regina variety seems to better tolerate stress conditions and light treatments with respect to the Mola variety. The next step will be the selection of the best conditions of growth for tomato seedlings, as light quality and intensity, to improve crop productivity and stress resilience thanks to the sustainable use of LED technology.

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P-68

Drought, salinity, and their combination affect the growth and secondary metabolites of tomato (*Solanum lycopersicum* L.)

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Keywords: antioxidant activities, carotenoids, drought, growth, phenolics, salinity, tomato

Climate change and global warming pose significant challenges to biodiversity and agricultural practices. These factors negatively affect agricultural productivity by enhancing environmental stresses, such as salinity and drought. An experiment was conducted to investigate the effects of drought, salinity, and their combination on the growth of tomatoes (*Solanum*

lycopersicum L.) and their secondary plant metabolites. The irrigation levels were defined as control (C): 25%, D1: 12.5%, and D2: 6.25% of soil weight. The salinity treatments included S1: 0.5% NaCl and S2: 1.0% NaCl, in addition to combinations of these factors. Growth parameters such as height, stem diameter, fruit weight, yield, and the number of leaves and fruits were measured. Secondary plant metabolites, including phenolics (chlorogenic acid, quercetin-3-rutinoside, and naringenin chalcone), carotenoids (lycopene, β-carotene, and lutein), and antioxidant activity (TEAC, DPPH, and TPC) were also assessed.

Severe drought conditions, accounting for 6.25% of soil weight, reduced plant height, fruit number, and yield, while concentration of lutein, chlorogenic acid, and naringenin chalcone increased under these conditions, as well as antioxidant activity (DPPH). Severe salt conditions (1.0% NaCl) increased antioxidant activity (DPPH) and total phenolic compounds, but did not affect plant morphology, specific phenolic compounds, lycopene, and β-carotene. Additionally, a significant interaction was observed between drought and salinity affecting β-carotene, total phenolic compounds (TPC), and antioxidant activity (TEAC). Moreover, there was no interaction between drought and salinity regarding plant morphological characteristics and specific phenolic compounds in tomato fruit. In contrast, a combination of severe conditions (6.25% soil weight and 1.0% NaCl) and moderate conditions (12.5% soil weight and 0.5% NaCl) resulted in higher β-carotene concentrations and antioxidant activity (TEAC). The increase in the concentration of health-beneficial compounds is encouraging for human nutrition, especially given the challenges posed by climate change. Under severe drought conditions, the yield of tomato plants was reduced by approximately 30%, whereas it remained unaffected under severe salinity.

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P-69

Climate change related lessons learned from a long-term field experiment with maize

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Keywords: planting date, genotype, fertilization, climate change, adaptation strategies

Maize is the second most important cereal crop in European agriculture and a widely used raw material for feed, food and energy production. Climate change studies over Europe predict a significant negative change in maize production. Finding appropriate and feasible adaptation strategies is a top priority for agriculture in the 21st century. Long-term agricultural experiments provide a useful resource for evaluating biological, biogeochemical, and environmental aspects of agricultural sustainability and for predicting future global changes. The objective of the study was to analyze a 30-year period of a multi-factorial (Variety × Fertilization × Planting date) long-term experiment at Martonvásár (Hungary) searching for traces of climate change as well as for favorable combinations of agro-management factors that can be used as adaptation options in the future.

The experiment involves: four planting dates (the first, second and third decades of April and first decade of May), five fertilization doses (0, 60, 120, 180 and 240 kg ha^{-1} N), and five varieties each year. While the choice of varieties changed over the years to reflect breeding progress, five different varieties were sown each year. Groups of early (FAO 290–320), medium (FAO 330–420) and late (FAO 430–550) varieties were established according to their FAO number. To analyze and extrapolate the data both in space and time, a multivariate statistical (response surface) model was used.

The results of the study yielded the following conclusions: (1) intensification of fertilization would not promote sustainable development in the region; (2) late hybrids (FAO number > 400) have no perspective in the Pannonian climatic zone and (3) earlier planting (first decade of April or even earlier) may become an effective adaptation option in the future.

It is essential to facilitate effective knowledge transfer to encourage farmers to adopt the proposed new practices. Implementing these anticipated beneficial measures, such as earlier planting and planting early hybrids, is expected to enhance the region's competitiveness and export capacity, ensuring sustainability in the future.

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P-70

Stomatal plasticity supports resilience of *Myrsine coriacea* to environmental changes in the Brazilian atlantic forest

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Keywords: water-use efficiency, adaptation strategies, altitudinal gradient

Stomatal functionality is crucial for regulating gas exchange between leaves and the atmosphere, directly influencing photosynthesis and water-use efficiency. This study investigated the stomatal morphology and kinetics of *Myrsine coriacea* (Primulacea) in populations from an altitudinal gradient in the Brazilian Atlantic Forest [(657, 930, 1,229, and 2,019 m a.s.l. (meters above sea level)] cultivated in a common garden (914 m a.s.l.). Permanent slides of the leaf epidermis were prepared to analyze stomatal size and density. Stomatal kinetics was assessed by the conductance (g_s) measurements during light-dark-light transitions (1000 – 0 – 1000 µmol m^{-2} s^{-1}). Parameters such as the maximum g_s slope (Sl_{max}) and the time to 50% of steady state ($t_{50\%}$) were evaluated. The 657 m a.s.l. population exhibited higher stomatal density and smaller stomata, while the 2,019 m a.s.l. population did not maintain the stomatal characteristics observed in its natural habitat, indicating phenotypic plasticity in response to the common garden environmental conditions. These adjustments highlight the populations' plasticity as strategies for water conservation and CO_2 use optimization, critical for their resilience to climate change and environmental variability. Despite the morpho-anatomical differences, populations showed no significant variation in gas exchange capacity or maximum conductance, indicating a similar gas exchange potential. Plants from higher altitudes (2,019 m a.s.l.) exhibited slower stomatal responses, both in opening and closing, which may limit CO_2 assimilation and reduce water-use efficiency in environments with rapid light fluctuations due to their water-conservation strategy. The 930 m a.s.l. population demonstrated excellent stomatal control, especially under intermittent light conditions, with a high capacity for rapid stomatal closure to conserve water. The 1,229 m a.s.l. population adopted a more conservative strategy, with low stomatal conductance and reduced sensitivity to light stimuli. These findings highlight the importance of further investigating the interaction between morphophysiological traits and environmental factors to predict species resilience under climate change scenarios.

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P-71

Molecular dynamics of chloroplast membranes and *COR14b* gene expression in cold-acclimated and deacclimated barley *HvBRI1* and *HvCPD* mutants. Relation to plant frost tolerance

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Keywords: chloroplast membranes, brassinosteroids, COR14b, EPR, frost tolerance

Cold acclimation (CA, cold hardening) is the process that prepares plants to survive winter conditions, including frost temperatures. A climate warming favours the emergence of deacclimation (DA, dehardening) caused by

periods of elevated temperatures that interrupt natural hardening processes, resulting in a reduction in frost tolerance of plants. The mechanisms of CA are quite well understood and involve many metabolic changes, including changes in the protein accumulation (i.a. cold-responsive proteins [CORs]), water management or hormones homeostasis. Cell membranes also play an important role in CA - an increase in membrane fluidity in the cold is a necessary condition to ensure proper membrane function. The physicochemical properties of membranes are determined mainly by their fatty acid composition but can also be modified by the incorporation of various compounds from groups of carotenoids, tocopherol and steroids (e.g. brassinosteroids [BR]). In comparison to CA, DA mechanisms are much less known.

The aim of this study was to answer the questions: (1) how do the phenomena of CA and DA affect the molecular dynamics of the chloroplast membranes of barley BR-mutants, (2) can any changes in molecular dynamics be linked to the level of BR and carotenoid accumulation in these membranes? (3) whether and how does the *COR14b* expression change? In addition, the study determined changes in the frost tolerance of barley plants subjected to CA and DA.

The study object was barley (Bowman cultivar and BR perception/biosynthesis impaired mutants (BW312, BW084). Plants, after growth at 20°C, were subjected to CA (3 weeks, 4°C) followed by DA (1 week, 14/9°C (d/n)).

Chloroplast membranes of mutants after CA showed greater fluidity (molecular dynamics) than under control conditions. After CA, higher fluidity of the chloroplast membranes of the mutants, relative to the Bowman cultivar, was also observed. DA of the mutants was associated with a rigidification of chloroplast membranes relative to membranes obtained from cold-acclimated plants. This phenomenon was less severe in the Bowman cultivar. The greater membrane fluidity in the cold-acclimated mutants may be one of several reasons favouring their higher frost tolerance (compared to the Bowman cultivar). At the same time, the increased accumulation of COR14b protein after DA may also be beneficial for maintaining the higher frost tolerance of the mutants (compared to the Bowman cultivar).

P-72

Response to drought stress in traditional varieties of durum wheat

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Keywords: drought stress, durum wheat, traditional varieties

The Mediterranean region is one of the most vulnerable areas due to climate change scenarios, and abiotic stresses such as drought are predicted to increasingly impact crop productivity, as is the case for durum wheat.

Considering these challenges, it is crucial to exploit germplasm material, which could represent a source of resilience to face major environmental variations. In this study, we used a panel of traditional varieties grown under different water regimes and performed phenotypic and transcriptomic studies to highlight possible differences in the response to drought among genotypes. High-throughput phenotyping was carried out throughout the life cycle of the plants, while flag leaves and developing grains were collected at 10 and 20 days after pollination (DAP) for RNA extraction and sequencing. Plant growth followed a Weibull curve under control conditions and a bell-shaped curve under stress conditions. Among the analyzed genotypes, we identified a few promising ones, showing a higher green area and delayed senescence, while exhibiting a smaller reduction in kernel weight, compared to Svevo cultivar, under drought conditions.

The integration of data from different omics approaches will reveal both common and unique mechanisms adopted by the distinct varieties in response to drought, contributing to a better understanding of the biological processes underpinning stress tolerance. This information will be critical for developing strategies to improve crop resilience and support agricultural productivity under changing environmental conditions.

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P-73

Responses to various environmental stresses of two different *Eruca* ecotypes grown under the same controlled conditions

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Keywords: Eruca sativa ecotypes, climate changes, adaptation mechanisms

Rocket, (*Eruca sativa*) is widely distributed all over the world. Most frequently its fresh leaves are consumed which contains a lot of secondary metabolites with antioxidant activity. The aim of the present work was to investigate whether two different ecotypes of *Eruca* (one of them acclimated to the Mediterranean and the other to the desert climate) have similar or different acclimation strategies to various abiotic stresses under the same controlled conditions.

Plants were grown in pots for 60 days under controlled conditions in a plant growth chamber (20/18 °C; 8/16 h light/dark periodicity; PPFD: 250 µmol m^{-2} s^{-1}). At the end of this period half of the plants were exposed to various abiotic stresses: moderate UV-B radiation (430 µW cm^{-2}) for seven days, low (5°C, 7 days) or elevated (35°C, 3 days) temperature and drought stress for 7 days. After the stress period physiological parameters were measured and samples were collected for biochemical analyses.

For detection of the severity of the stress chlorophyll-a fluorescence parameters were measured. A small decrease was found in the F_v/F_m parameter of the older leaves of desert plants but the quantum yield of PSII did not change during the moderate UV-B stress. Changes in the flavonoid and glucosinolate contents were also investigated. 59 significantly differentiated glucosinolates were found and 45 were identified during the measurements. More than 20 compounds of flavonoids and plant hormones were also measured and the amino acid, sugar contents and the compounds of TCA cycle were also determined. From these results we could see that the metabolic changes in two ecotypes of *Eruca* were different during abiotic stresses.

P-74

Integration of multi-omics data from core sweet potato germplasm in Taiwan and the development of digital breeding platforms

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Keywords: sweet potato, multi-omics, image recognition, digital breeding

Sweet potato is an important staple crop with high nutritional value and broad adaptability, making it a key food source in many regions and playing an indispensable role in addressing global food security and nutritional challenges. The sweet potato germplasm bank at the Chiayi Agricultural Experiment Branch of the Taiwan Agricultural Research Institute has collected and preserved approximately 1,435 sweet potato lines and varieties, primarily maintained through vegetative propagation via tissue culture. This method of preservation has resulted in high genetic uniformity among genotypes. To address the challenges posed by climate change, establishing a core germplasm collection is essential to exclude genetically identical or highly similar materials, thereby preserving a subset with greater genetic diversity. This approach will reduce the labor and time required for maintenance and breeding. Our team aims to integrate multi-omics data–including genomics, transcriptomics, and phenomics–to develop an optimized data integration algorithm. This system is further enhanced by image recognition technologies, which support Taiwan's digital sweet potato breeding strategy. For the multi-omics component, data will be manually collected from core germplasm and key

cultivars (e.g., Tainung No. 57, No. 66, and No. 73) grown in experimental fields. The data will then undergo integrative analysis using systems biology-based computational frameworks. For the image recognition component, surface traits of sweet potatoes will be extracted and analyzed using image analysis techniques combined with deep learning. The resulting data will be uploaded to a cloud-based database. The outcomes of this research will support trait selection and key gene identification in Taiwanese sweet potato breeding programs. In the future, the system may enable real-time field monitoring by agricultural research units or farmers through web or mobile platforms, embedding machine learning into agriculture to enhance productivity while reducing labor costs and resource waste. Ultimately, this work aims to advance the digital transformation of sweet potato breeding in Taiwan.

P-75

Functional roles of sHSPs in thermotolerance and heavy metal stress resistance in rice

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Keywords: small heat shock proteins (sHSPs), genetic engineering, transgenic rice, thermotolerance, heavy-metal toxicity

Small heat shock proteins (sHSPs) serve as molecular chaperones that assist in properly folding and refolding proteins, thereby preventing damage induced by stress. While sHSPs are well-known for their role in thermotolerance, they also respond to various environmental stressors, including heavy metal toxicity and oxidative stress. This study examined the distinct functions of two closely related rice sHSPs, OsHsp16.9A and OsHsp18.0, in thermotolerance and resistance to heavy metal stress. Transgenic rice lines overexpressing either OsHsp16.9A (OsHsp16.9A-OE) or OsHsp18.0 (OsHsp18.0-OE) were generated under the control of the ubiquitin 1 (Ubi1) promoter. Under high- temperature stress, OsHsp16.9A-OEs show a higher seed germination rate than the wild-type (WT) controls and OsHSP18.0-OEs. After exposure to copper (Cu) and cadmium (Cd), the OsHsp18.0- overexpressing lines exhibited significantly higher chlorophyll retention and lower malondialdehyde (MDA) accumulation compared to wild-type (WT) and OsHsp16.9A- overexpressing lines. Furthermore, the OsHsp18.0-OE lines demonstrated reduced electrolyte leakage and enhanced cell membrane integrity under Cu and Cd stress. Biochemical analyses revealed that these lines exhibited significantly elevated activities of catalase (CAT) and ascorbate peroxidase (APX) under Cu and Cd stress conditions, indicating their role in scavenging reactive oxygen species (ROS). These findings suggest that OsHsp18.0 plays a critical role in heavy metal tolerance, potentially through protein-protein interactions similar to the OsHsp16.9A-OsHsp101 complex in thermotolerance. In summary, the distinct roles of OsHsp16.9A in thermotolerance and OsHsp18.0 in heavy metal stress tolerance highlight their potential for genetic engineering to improve crop resilience and agricultural productivity under environmental stress.

P-76

Gas exchange and ionome profiling across multiple tissues and cell types under salt stress: Distinct tolerance strategies in faba bean (*Vicia faba*) and maize (*Zea mays*)

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Faba bean (C_3) and maize (C_4) exhibit contrasting salt-tolerance strategies due to differences in stomatal complex composition and morphology. To dissect these mechanisms, two genotypes per species - faba bean (sensitive Fuego, tolerant Scoop) and maize (sensitive LG30222, tolerant ES-Metronom) - were grown under controlled conditions and exposed to six treatments: control, 50 mM NaCl, 100 mM NaCl, 50 mM Na_2SO_4, 50 mM $CaCl_2$, and PEG6000-induced osmotic stress. Gas exchange was measured in fully expanded leaves, and ionome profiling was conducted on leaves, peeled epidermis (faba bean), or whole leaves (maize) using ICP-OES/MS and Cryo-SEM-EDX.

PCA showed species- and genotype-specific responses. Scoop (Tolerant) shows greater responsiveness to mild NaCl, indicated by a larger shift (distance to origin), suggesting an early proactive adjustment to maintain ion homeostasis. Fuego (Sensitive) responds more under PEG, showing a delayed or reactive adjustment strategy, characterized by accumulation of leaf K and stomatal Mg, potentially as a stress-coping mechanism rather than

long-term tolerance. In maize, tolerant ES-Metronom uses a proactive strategy with early ion regulation and osmotic adjustment (GC Cl^-, stomatal complex Ca^{2+} under PEG), while sensitive LG30222 shows a reactive strategy, with greater stomatal Cl^- accumulation under Na_2SO_4 and PEG, indicating weaker ion homeostasis. ES-Metronom also sequestered Na^+/Cl^- in subsidiary cells, preserving gas exchange, a strategy absent in LG30222.

Faba bean tolerance relies on early ion homeostasis, while maize depends on osmotic adjustment, ion cellular compartmentalization and gas exchange moderation. These findings highlight distinct salt-adaptation strategies between C_3 legumes and C_4 cereals, informing stress-resilient crop breeding.

P-77

Post-El Niño drought effects on the functional traits and community structure of atlantic forest trees in Brazil

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Keywords: leaf traits, leaf area, restinga

Drought periods can induce significant shifts in functional traits and plant community structure, favoring species with high phenotypic plasticity (generalist species). Leaf traits are key indicators for understanding the impacts of climate change on tropical forests. This study assessed temporal changes in species and functional diversity of restinga plant communities nearly a decade after the severe drought caused by the 2015-2016 El Niño. A phytosociological survey was conducted, and leaf samples were collected from 80% of the most abundant tree species in a plant community at Paulo César Vinha State Park, Guarapari, ES, Brazil, in 2016 and 2024. We measured several leaf parameters, such as leaf thickness (LT), specific leaf area (SLA), succulence (SUC), leaf dry matter content (LDMC), and leaf tissue density (TD), as well as phytosociological descriptors, including basal area, density per hectare, Shannon-Wiener index, effective richness, and mortality rates. Comparisons between the two periods revealed subtle structural changes driven by the recruitment of dominant species and more pronounced shifts in generalist species traits. A slight increase in basal area and density per hectare was observed, while the mortality rate remained constant. By 2024, plants exhibited reduced SLA, increased LT, and lower TD, indicating improved water retention and nutrient use efficiency. These findings suggest that restinga plant communities are adapting to water-limiting conditions, favoring drought-resistant and tolerant phenotypes, such as generalist species.

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DIALOGUE WITH SOCIETY

P-78

Discovery, understanding, engagement: Opportunities for research-based learning in plant biology education

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Keywords: research-based learning, experiential learning, bryophytes

During the instruction of botany and plant physiology, students often remain passive recipients of theoretical knowledge, while the complexity of plant functions and the dynamics of their living systems become only partially perceptible to them. Experiential learning, particularly through inquiry-based learning (IBL) and research-based learning (RBL), provides an opportunity for students to deepen their understanding through active experimental and observational activities based on their own questions. This approach allows students to explore the physiological and morphological characteristics of plants in relation to their own inquiries. This method can be particularly effective with simpler yet model organisms, such as bryophytes which are often overlooked in educational settings. The application of research-based education in these instances fosters the development of students' scientific thinking, enhances their engagement with the subject matter, and supports the growth of skills such as hypothesis formulation, data collection, interpretation, and argumentation. Our aim is to demonstrate how lower taxonomic groups of plants–such as mosses (*Physcomitrium patens*, *Syntrichia ruralis*), ferns (*Polypodium sp.*), and certain algae (*Spirogyra sp., Chara sp.*)–can serve as effective tools for discovery-based learning. These organisms are ideal not only due to their simple structure and rapid life cycles but also because they respond dramatically to changes in environmental factors, thus providing opportunities for real-time observations and drawing conclusions.

GENOMICS AND EPIGENOMICS

P-79

Investigation of the diverse roles of RNA-directed DNA methylation in barley

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Keywords: argonautes, small RNA, RdDM, Barley, RISC

RNA-Directed DNA Methylation (RdDM) is unique, plant specific, regulatory system controlling DNA methylation of specific DNA sequences. RdDM is required for maintaining genome stability and transcriptional repression of various genes. RdDM involves the production of small interfering RNAs (siRNAs), typically 24 nucleotide long, via the action of DICER- LIKE 3 (DCL3) enzyme that cleaves double stranded RNA precursors. SiRNAs are then loaded into effector proteins, ARGONAUTE (AGO)4 or 6 forming RNA-induced silencing complexes (RISCs) which mediate sequence specific DNA methylation.

The aim of our work is to investigate the biological roles of RdDM pathway in an economically important monocot plant, barley, possessing an extremely large genome. Preparing various genome edited mutant and transgenic over-expressor/reporter barley lines we investigated the specific biological role of selected RdDM protein components. We found that in barley, in contrast to model Arabidopsis system where RdDM mutans exhibit only minor phenotypic alterations, RdDM is responsible for controlling vegetative and reproductive processes, such as seed development and also plays role in alleviating heat stress damages. Our findings can open a new avenue for understanding of basic biological regulatory processes of RdDM in an important monocot crop plant and this knowledge might be suitable for enhancing crop quality in the near future.

P-80

Widespread alternative transcription start site selection in Norway spruce results in tissue specific proteofoms

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Keywords: Transcription start sites, spruce, TSS-sequencing, needle, wood

The adaptation of perennial trees in temperate ecosystems relies on their remarkable plasticity, governed by a combination of endogenous and environmental factors. Until now, our understanding of these endogenous factors and their molecular mechanisms has been limited. In plants, gene expression is tightly controlled through various transcription-dependent RNA quality control pathways, with transcription initiation being a key regulatory step. Thus, understanding the dynamics of transcription initiation, the signals that specify it, and the underlying molecular mechanisms is crucial. In this study, we performed a genome-wide analysis of transcription start sites (TSSs) in Norway spruce, Picea abies, using Transcription Start Site-sequencing (TSS-seq). Our findings reveal unannotated TSSs on both sense and antisense strands at single nucleotide resolution. By clustering the TSSs into clusters (TCs), we identified alternative transcriptional initiation (ATI) sites in numerous genes, highlighting variability in transcription initiation. Moreover, we discovered that different tissues in spruce utilize widespread tissue specific start sites, giving rise to tissue specific protein isoforms. These findings provide a comprehensive overview of TSS usage as a critical endogenous control factor and can in part explain the exceptional plasticity in spruce.

P-81

Gene co-expression analysis identifies candidate epigenetic regulatory modules of periderm development

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Keywords: cork, DNA methylation, histone modifications

The periderm, a protective tissue formed during secondary growth, acts as a defence barrier against environmental stress. In cork oak (*Quercus suber* L.), a long-lived phellogen (cork cambium) generates a thick, suberized cork layer with significant ecological and economic value. While epigenetic regulation of periderm development has been suggested (reviewed in Inácio et al., 2022) , the underlying genetic components remain largely uncharacterized.

Here, we identify candidate epigenetic regulatory modules of periderm development in *Quercus suber* through a targeted co-expression analysis of 89 publicly available RNA-seq libraries, primarily from stem and root tissues. Normalized raw read counts were used to compute gene co-expression, focusing on 134 epigenetic regulators associated with DNA methylation/demethylation, histone modifications, and chromatin remodelling.

Co-expression analysis was conducted applying 12 correlation methods, and a final network resulting from the aggregation of all correlation methods was obtained, representing selected epigenetic markers with strong co-expression with 3219 candidate target genes. Histone methyltransferases showed the higher number of direct interactions, while DNA methylation and demethylation the lowest number. Interestingly, histone demethylases showed strong co-expression with genes related to meristem activity and cell fate specification. While further analysis of specific interactions is ongoing, these

results provide new insights into the epigenetic control of periderm formation in cork oak and lay the groundwork for future functional validation.

This work was supported by the EpiCork project (DOI: 10.54499/PTDC/ASP-SIL/1717/2020).

Inácio, V., Santos, R., Prazeres, R., Graça, J., Miguel, C. M., & Morais-Cecílio, L. (2022). Epigenetics at the crossroads of secondary growth regulation. Frontiers in Plant Science, 13. https://doi.org/10.3389/fpls.2022.970342

P-82

Identification of a *cp-2* gene controlling compact plant architecture in cucumber

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Keywords: compact phenotype, *Cucumis sativus* L., plant growth architecture

Cucumber plants have indeterminate, long, trailing stems that require a large area for planting and high labor inputs for management. Therefore, identifying genes responsible for compact growth is of significant value in cucumber breeding focused on improving plant growth architecture. Compact, semi-dwarf, or dwarf growth habits offer several agronomic advantages, such as higher planting density, reduced water usage, and easier harvesting, contributing to higher yields and cost savings. Here, we report the research progress on cucumber line L504, characterized by compact growth habit controlled by a single recessive gene *cp-2*, described by Kubicki et al. (1986). A segregating $F_{2:3}$ population was developed from a cross between the wild-type line L500 and the compact line L504. A genomic region associated with compact plant growth was identified on chromosome 4 through phenotyping and high-throughput genotyping. Fine mapping narrowed this region, and a candidate gene for *cp-2* was identified. Subsequent analysis revealed that this gene encodes a protein of unknown function, containing a serine/threonine kinase domain. In addition, RNA-seq analysis identified 641 genes differentially expressed in the compact line, and RT-qPCR showed upregulation of the candidate gene in various organs. These findings provide new insights into the molecular mechanisms underlying the compact growth of cucumber and offer potential targets for genetic improvement.

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P-83

DNA methylation regulates ageing in monocarpic plants

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Keywords: DNA methylation, ageing, barley, tomato

Biological ageing is a complex, multifactorial process observed across the tree of life. Understanding how and why organisms age is a fundamental question with broad application, including organism performance and disease treatment. DNA methylation dynamics are a key aspect of biological ageing and have recently been modelled to predict chronological age in vertebrates. Although evidence suggests that DNA methylation patterns may describe plant age, predictions of plant age-dependent epigenetic changes are still in the early stages of research. As plants continuously form new organs from meristems, an important question arises whether plant age and organ ageing are coupled in monocarpic plants.

Here, we present our research on the leaves aging process of two monocarpic plant species: barley (*Hordeum vulgare*) and tomato (*Solanum lycopersicum).* Our recent studies allowed us for the first time to identify and characterize 117 genes involved in epigenetic processes in barley, especially in terms of control of age-dependent changes in plant leaves. Current results underlie an important role of DNA methylation in regulating barley leaf ageing. Moreover, detailed phenotyping of tomato RNAi plants with different DNA methylation status, namely hypomethylated *met1* and hypermethylated *dml* plants, shows much increased ageing in *met1* lines compared to WT plants, while *dml* lines show a juvenile phenotype.

Our results suggest that proper maintenance of DNA methylation pattern is an important player regulating barley- and tomato leaf ageing process. However, the exact mechanisms underlying the observed phenomenon require further investigation. Moreover, it would be the most interesting to reveal if this observation is more universal and refers to another monocarpic plant species as well, as it has been recently suggested for rice and Arabidopsis.

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P-84

RT-qPCR analysis of candidate genes related to self-incompatibility in *Astragalus membranaceus*

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Keywords: *Astragalus membranaceus*, self-incompatibility, medicinal plant

Astragalus membranaceus Bunge, a perennial herbaceous species in the Leguminosae family, is widely distributed across East Asia–including China, Korea, and Japan–and is traditionally used for medicinal purposes. Botanically, this species exhibits self-incompatibility (SI), a key reproductive strategy in angiosperms that prevents self-fertilization and promotes genetic diversity. The present study aimed to identify candidate genes associated with SI in *A. membranaceus* and to verify their expression patterns using reverse transcription quantitative PCR (RT-qPCR).

Four breeding lines exhibiting SI traits (Am011, Am017, Am019, and Am023) were selected. Floral tissues were sampled at four different flowering stages from July to October, with three biological replicates per stage. Total RNA was extracted, quality-checked, and used for cDNA synthesis. Among 20 differentially expressed genes (DEGs) selected as SI-related candidates, 16 were used for primer design and RT-qPCR analysis. Temporal expression profiles of these genes were assessed to identify expression patterns associated with SI during the flowering period.

RT-qPCR analysis revealed that Am04G017760 exhibited significant temporal variation in expression levels. This gene showed low expression in July and August, followed by a gradual increase from September, peaking in October. These findings suggest that SI mechanisms in *A. membranaceus* remain active during late flowering stages, with Am04G017760 potentially playing a regulatory role at the molecular level. Further comparative expression studies between lines with strong and weak SI expression are warranted. If validated, Am04G017760 may serve as a valuable molecular marker for SI-based lineage classification and breeding applications in *A. membranaceus*.

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P-85

Paleo-polyploidization in *Hibiscus syriacus* adapting to changing climates during evolution

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Rose of Sharon (*Hibiscus syriacus*) is one of the most widespread garden shrubs globally and has a complex genome structure resulting from multiple rounds of polyploidization events during its diversification and evolution. Here, we report two reference genomes for *H. syriacus* varieties, Baekdansim and Gangneung, with assembly sizes of 1.94 Gbp and 1.99 Gbp, respectively. Synteny-based analysis revealed consistent chromosome pairing patterns across both varieties, including six sets of syntenic relationships among three chromosomes, two sets among four chromosomes, two sets among five chromosomes, and one set among six chromosomes. These findings indicate that extensive chromosomal rearrangements occurred during consecutive diploidization processes. The observed syntenic patterns provide strong support for the hypothesis that these varieties have undergone diploidization from a dodecaploid ancestor, with extensive unequal chromosome pairing and rearrangement playing a key role in their genomic evolution.

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P-86

ISSR and cpDNA analyses to assess genetic diversity status of the endangered giant plantain (*Plantago maxima* Juss. ex Jacq.) in Hungary

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Keywords: conservation, distribution edge, ex situ, threatened, small populations

Plantago maxima Juss. ex Jacq. is a perennial species distributed along the Eurasian steppes zone. The range of the species extends through East Europe to West Asia. The westernmost range edge of the species' distribution is located in Hungary where it is represented by four, fragmented and highly endangered populations. In Europe - beside the Hungarian populations - the species has one more isolated locality in Bulgaria. However, the species has a least concern threat status worldwide, in the EU it is considered endangered. We consider that protecting gene stock of the species on its edge of its distribution is vital, therefore in 2015 an *ex situ* stand was established in the Botanical Garden of Soroksár. Population diversity using ISSR and cpDNA markers of all Hungarian range edge, natural populations were evaluated, and one established *ex situ* population was also included. One population from the centre of distribution (Kazakhstan) was implemented in the cpDNA haplotype study to compare the peripheral vs. central populations' haplotype diversity. Meanwhile we studied the performance of the *ex situ* stands during 2022 and 2023 (eight years after *ex situ* establishment).

Our study revealed that even though the Hungarian populations are small and fragmented, they reach a relatively high genetic diversity (mean value of H_e = 0.220). It can be reasoned by additional gene flow between the populations and recent isolation events. The AMOVA results revealed that most of the diversity occurs within populations (92%), which is in accordance with STRUCTURE results. Genetic diversity of *in situ* populations and *ex situ* stock was very similar, and in some stands displayed the same values. This result is important, showing the genetic representativeness of the *ex situ* stock, which is an important criterion for long-term conservation. Comparative field observations have shown that although the number of individuals in *ex situ* decreased after introduction, flowering and seed production started successfully and viable seeds were produced. The hygrophilous *ex situ* stand appeared to be the most suitable, with individuals exhibiting similar leaf traits to those from Kakucs *in situ* stand from where the seeds originated.

Our study revealed the presence of a distinct haplotype preserved in the Hungarian populations, compared to the Kazakhstan population. Even different markers and more samples should be analysed and implemented to estimate the distance between Central-Asian and European populations in the future.

P-87

Establishing a genomic selection pipeline for the long-term enhancement of tomato heat tolerance

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Keywords: heat tolerance, genomic selection, fruit number

Heat stress poses a critical challenge to tomato production, particularly in tropical regions, where elevated temperatures substantially diminish yields. Over the past half-century, the World Vegetable Center has pursued tropical tomato breeding strategies, generating novel genetic resources and employing field trials to enhance heat tolerance. Heat tolerance is a complex trait; fruit number, significantly affected by heat stress, is the primary yield component in open-field cultivation. In this study, we employed advanced image-based phenotyping methods to enhance monitoring and data collection in open-field tomato yield trials. Leveraging YOLOv9, we achieved an average precision of 77% and a recall rate of 84% in fruit number estimation. We also investigated the performance of genomic selection models by systematically varying single

nucleotide polymorphism (SNP) density and sample size, using a training population derived from diverse pedigrees. Our findings indicate a positive correlation between SNP density, sample size, and prediction accuracy, with accuracy plateauing at 0.8 when approximately 30,000 SNPs were included. Notably, increasing the sample size continued to improve predictive accuracy beyond this plateau. These results provide a robust foundation for the application of genomic selection strategies aimed at enhancing tomato heat tolerance. Future work will expand the dataset to multiple years and incorporate climate variables, thereby refining model accuracy and strengthening the long-term impact of genomic selection on heat stress resilience in tomato breeding.

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P-88

PharaohFUN: PHylogenomic Analysis foR plAnt prOtein History and FUNction elucidation

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Keywords: phylogenomics, *Viridiplantae*, transcription factors

Since DNA sequencing has become commonplace, the development of efficient methods and tools to explore gene sequences has become indispensable. In particular, despite photosynthetic eukaryotes constituting the largest percentage of terrestrial biomass, computational functional characterization of gene sequences in these organisms still predominantly relies on comparisons with *Arabidopsis thaliana* and other angiosperms. This work introduces PharaohFUN, a web application designed for the evolutionary and functional analysis of protein sequences in photosynthetic eukaryotes, leveraging orthology relationships between them.

PharaohFUN incorporates a homogeneous representative sampling of key species in this group, bridging clades that have traditionally been studied separately, thus establishing a comprehensive evolutionary framework to draw conclusions about sequence evolution and function. For this purpose, it incorporates modules for exploring gene tree evolutionary history, expansion and contraction events, ancestral states, domain identification, multiple sequence alignments, and diverse functional annotation. It also incorporates different search modes to facilitate its use and increase its reach within the community. Tests were performed on the whole transcription factor toolbox of *Arabidopsis thaliana* and on CCA1 protein to assess its utility for both large-scale and fine-grained phylogenetic studies. These exemplify how PharaohFUN accurately traces the corresponding evolutionary histories of these proteins by unifying results for land plants, streptophyte and chlorophyte microalgae. Thus, PharaohFUN democratizes access to these kind of analyses in photosynthetic organisms for every user, independently of their prior training in bioinformatics.

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P-89

Cold acclimation - a tug of war between transcriptional activation and repression

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Keywords: cold acclimation, transcriptional regulation, non-coding RNA

Climate change is driving increasingly extreme weather conditions and rapid temperature fluctuations. As sessile organisms, plants must rely on intricate regulatory mechanisms to cope with environmental stress. One key pathway involved in cold stress adaptation is the CBF-dependent signalling pathway, where C-repeat/dehydration-responsive element binding factors (*CBFs*) serve as central regulators. Two of the *CBF* genes, *CBF1* and *CBF3*, sit next to each other in the Arabidopsis genome and both genes are tightly controlled to ensure an optimal response to stress.

One regulatory mechanism involves the antisense long non-coding RNA *SVALKA* (*SVK*), which downregulates *CBF1*. *SVK* is activated a few hours after cold exposure, leading to RNA Polymerase II stalling and the premature release of immature *CBF1* mRNA. Interestingly, *SVK* appears to be regulated by an upstream transposable element. However, the methylation status of this transposable element does not change during cold acclimation, raising the question of how exactly it contributes to *SVK* regulation.

Beyond *SVK*, we have identified another long non-coding RNA, *SVALNA* (*SVN*), as a negative regulator of *CBF3*. Preliminary results and the positional conservation between *SVK-CBF1* and *SVN-CBF3* suggest a common mechanism. Understanding how *SVN* and *SVK* function together or independently to fine-tune the *CBF* response will provide deeper insights into the complexity of cold stress regulation.

While *SVK* and *SVN* act as repressors of *CBF*s, we are also exploring novel activators of *CBF* transcription, particularly ARGONAUTE 1 (AGO1). AGO1 is a highly conserved protein known for binding small non-coding RNAs (sRNAs) of 21–24 nucleotides, traditionally associated with post-transcriptional gene silencing in the cytosol via RNA interference. However, recent evidence suggests that AGO1 also functions in the nucleus, binding chromatin. After cold exposure, we observed increased AGO1 binding at the *CBF* genomic region, along with an AGO1-bound sRNA cluster in the *SVK* regulatory region, suggesting a coordinated regulation of AGO1 and non-coding transcription on *CBF1* and *CBF3*.

Understanding how AGO1, *SVK*, and *SVN* coordinate cold stress responses could provide valuable insights into plant resilience and inform strategies to enhance stress tolerance across diverse plant species.

P-90

Omics-based gene discovery and regulation of centelloside biosynthesis

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Keywords: *Centella asiatica*, chromosome-scale genome assembly, centelloside

Centellosides, triterpenoid saponins found in *Centella asiatica*, are highly valued for their pharmaceutical and cosmetic applications. In South Korea, centelloside production primarily relies on imported dried leaves of *C. asiatica*, while the utilization of domestically cultivated varieties remains limited. To enhance centelloside content in domestic *C. asiatica* varieties, it is crucial to establish a fully phased, high-quality genome assembly as a foundational resource. Here, we present the phased genome assemblies of three domestic *C. asiatica* varieties: Jaerae, Sunbal, and Pyungtaek. The assemblies comprise nine chromosomes, with a total genome size ranging from 439.8 to 450.6 Mb and an N50 value of 53.7 to 54.2 Mb. The BUSCO value ranges from 98.7% to 99.0%. Gene annotation identified 27,453 protein-coding genes with an average gene length of 3,551 bp. These high-quality genome assemblies provide a crucial foundation for identifying key enzymes involved in centelloside biosynthesis and facilitating future genomic breeding efforts. Furthermore, this study reports the first fully phased genome of *C. asiatica*, providing a valuable genomic resource for medicinal plant research and biotechnology.

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P-91

Self-regulation of trithorax and polycomb epigenetic complexes via H3K4me3 and H3K27me3 marks in *Arabidopsis thaliana*

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Keywords: TrxG/PcG complexes, histone marks, gene expression

Trithorax (TrxG) and Polycomb (PcG) are epigenetic factors that regulate gene expression. TrxG catalyzes the H3K4 trimethylation (H3K4me3) mark to active gene expression, while PcG represses transcription by deposition of the H3K27 trimethylation (H3K27me3) mark. Their activity depends on different proteins that "write", "read", and "erase" histone marks, ensuring stable and efficient regulation. These complexes are highly conserved across organisms including animals and plants, demonstrating their importance in gene regulation. In plants, both TrxG and PcG regulate gene expression of different developmental processes from embryogenesis to flower development. Their precise regulation also contributes to phenotypic plasticity, allowing plants to adapt to adverse environmental conditions.

While much is known about the function of TrxG and PcG proteins and the processes in which they are involved, little is known about how the genes encoding these proteins are transcriptionally regulated. In order to find out characteristics that may indicate autoregulation by their marks, we analyzed public datasets of gene expression and H3K4me3 and H3K27me3 enrichment in 38 genes involved in epigenetic regulation, including DNA methyltransferases, PcG and TrxG histone methyltransferases (HMTs), H3K4me3 and H3K27me3 readers, HMTs for H3K9me3, and histone demethylases.

We found out that most of the 38 genes are enriched in H3K4me3 at the TSS region while only a few genes were enriched with the H3K27me3 mark. Our analysis revealed differential regulation among genes within homologous functions. For example, of the three PcG HMTs *MEA, CLF, SWN*, only *MEA* showed H3K27me3 enrichment. Likewise, of two H3K27me3 readers, only *EMF1* showed H3K27me3 enrichment. Given that *MEA* is expressed only during embryogenesis and *EMF1* displays specific expression, we hypothesize that PcG autoregulation is tissue-specific. In contrast, TrxG HMTs were enriched by their own mark H3K4me3, suggesting TrxG autoregulation is widespread and not limited to specific tissues. Moreover, other genes that do not belong to TrxG and PcG such as DNA methyltransferases or H3K9me3 HMTs, showed low or no enrichment of H3K4me3 and H3K27me3, suggesting that could be regulated by other mechanisms. The Implications of TrxG and PcG self-regulation will be discussed.

HIGH THROUGHPUT PHENOTYPING AND REMOTE SENSING

P-92

Integrative phenotyping combined with modelling as a tool for predicting harvest-related traits

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Harvest-related traits are key agronomic traits for cereal crops. Understanding the influence of abiotic stress on yield and the ability to find the most predictive traits for final yield are important for understanding and characterizing crop resilience strategies. Using a high spatial and temporal phenotyping approach with multiple imaging sensors we monitored growth dynamics of plants and assessed their physiological responses throughout development until reaching the maturity stage under control and drought conditions. Here we present a dataset comprising 145 traits over 70 time points under two conditions for six barley lines including elite, cultivated, and wild lines. We applied advanced data analysis using machine learning approaches for effective integrative visualisation and subsequent modelling. The main objectives of this study were to identify distinct traits that differentiate drought-stressed from well-watered plants and to accurately predict harvest traits. Using a classification model, we observed an accurate separation between control and drought treatments and ranked the tolerance levels of different lines. For the harvest prediction models, we evaluated all time points under different watering regimes compared with models using only early time points and determined the importance of traits. Finally, we analysed the variance components of highly predictive traits and identified which traits are mostly driven either by genetic, environmental or time components. With the applied modelling approach, we were able to predict the harvest-related traits and pinpoint the most predictive traits at specific time points. Identifying predictive traits early in the developmental stage can help breeders find stress-tolerant traits of interest.

P-93

High-throughput analysis and sorting of intact plant protoplasts, seeds, pollen, and calli using COPAS VISION, FP, and BioSorter Instruments

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Keywords: high-throughput screening, flow cytometry, automation

Union Biometrica's large particle flow cytometers facilitate the analysis and gentle dispensing of large objects, including plant calli, pollen grains, plant protoplasts, and *Wolffia*. This study demonstrates the versatility of this technology by analyzing, selecting, and sorting a diverse range of samples using several large particle flow cytometry platforms. Subpopulation selection is achieved through parameters such as relative size, optical density, (auto-)fluorescence, and profiler data. Furthermore, the COPAS VISION system expands flow cytometry capabilities by incorporating a brightfield camera to capture images of samples as they pass through the flow cell. Dispensing can be performed into various receptacles, including conical tubes, Eppendorf tubes, and multiwell plates. Union Biometrica's large particle flow cytometry technology has proven to analyze and sort large objects with greater speed and precision compared to existing manual techniques. By automating these traditionally time-consuming manual processes, experimental timelines are significantly reduced, human error is eliminated, and previously unfeasible experiments become possible.

P-94

Development of a phenotypic assessment system for heat-tolerant tomatoes

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Tomato is a globally important vegetable crop, but its productivity is highly sensitive to elevated temperatures. High-temperature stress reduces photosynthetic efficiency, inhibits vegetative growth, and ultimately lowers yield. While previous studies have linked physiological responses during the reproductive stage with fruit production, traditional assessments of heat tolerance are often destructive, labor-intensive, and time-consuming. In this study, we investigated the relationship between vegetative-stage physiological responses and reproductive-stage fruit production under prolonged heat stress. We employed several non-destructive imaging and sensing techniques, including measurements of chlorophyll fluorescence (Y(II)), canopy temperature, leaf angle, and foliar reflectance spectra. Under high temperatures, most tomato varieties exhibited decreased Y(II), rapid canopy temperature adjustment, enhanced hyponasty, and reduced vegetation indices. Correlation analysis revealed significant relationships between these imaging-based phenotypic traits and both fruit set number and fruit set rate under heat stress. Stepwise regression further demonstrated that a combination of vegetation indices, canopy temperature, and leaf angle best predicted fruit set rate. Transcriptome analysis identified the involvement of potassium channel genes and chlorophyll-related genes in the phenotypic variation observed among varieties under heat stress. These findings suggest that integrating multiple image-based phenotyping indices provides an efficient, high-throughput approach for screening heat-tolerant tomato varieties. This method offers a practical and scalable alternative to traditional, destructive screening techniques for evaluating complex physiological traits associated with thermotolerance.

P-95

Complex phenomics in early growth phase of maize to support breeding of drought tolerant hybrids

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Keywords: maize, phenotyping, drought, stress adaptation

Improving crop resistence to extreme environmental conditions is a major goal of modern breeding programs. Phenotyping platforms have become essential tools in the breeding of crops like maize, enabling the rapid and precise measurement of various phenotypic traits. In this study, 14 maize pre-breeding hybrid lines were evaluated at early developmental stages using an automated phenotyping platform under greenhouse conditions. Plants were grown in plexiglass columns with automated irrigation set to 60% (control) and 30% (drought stress) of soil water capacity. A strong correlation (r = 0.70) between manually measured biomass and digitally estimated biomass confirmed the system's reliability. Based on biomass reduction under drought, hybrids were classified into Tolerant I, Tolerant II, and Sensitive groups. Sensitive hybrids showed the highest biomass under control conditions, while Tolerant groups maintained better performance under stress. Tolerant II hybrids combined high biomass under both conditions, whereas the biomass of the Tolerant I group plants showed the minimal reduction. The majority of the measured chlorophyll fluorescence parameters showed positive correlation with biomass, while a few were negatively correlated. Under stress, these correlations often reversed, varying by tolerance group. The development of the root system also differed significantly between the groups under both irrigation regimes, further differentiating the tolerance groups. Notably, drought stress led to reduced water consumption and altered correlations between root development, water use, and water use efficiency.

Based on comprehensive trait analysis, one hybrid from the Tolerant II group was identified as a promising candidate for breeding programs targeting regions susceptible to drought. This study highlights the utility of automated phenotyping platforms to detect complex traits that contribute to stress adaptation in crops.

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P-96

Effect of different boron concentrations on germination dynamics of sugar beet (*Beta vulgaris* L.)

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Keywords: sugar beet, boron stress, germination

The presence of boron (B) directly influences the development and physiological condition of sugar beet plants (Huo et al., 2022), therefore it deserves special attention in terms of supply of this important microelement. B is essential for many processes in plants, but the concentration range required for normal plant growth is narrow. This study represents the initial phase of a longer series of investigations, aiming to assess the effects of different B concentrations on germination dynamics, in order to determine the concentration at which boron becomes toxic to sugar beet germination.

To assess this, sugar beet seeds were treated with different boric acid (H_3BO_3) concentrations. Our treatments were 50 µM, 100 µM, 500 µM, 1000 µM and 2000 µM H_3BO_3, and distilled water was used in the control group. The main germination indices used in our work are germination percentage (GP), germination energy (GE), mean germination time (MGT), and germination time index (GSI).

Our results demonstrated that boron treatments of 100 and 500 µM had a positive effect on sugar beet germination, which was reflected in both the germination dynamics and the shoot length of the seedlings, which was on average 35.8 and 22.6 mm longer, than the third highest treatment. The highest GP was 95% for 500 uM, and the shortest MGT (2.11) was also observed in this group. However, the 2000 µM treatment improved germination parameters, but showed signs of oxidative stress, as indicated by reduced total antioxidant capacity. In previous work of Song et al. (2019) toxic (2775 uM) concentrations of boron manifested increases of superoxide dismutase, peroxidase, catalase, malondialdehyde in sugar beet leaves, that indicates depletion of non-enzymatic antioxidants, which was probably the case in the present experiment, as indicated by the concentration-dependent decrease of FRAP values and germination parameters. Future investigations will focus on the physiological and molecular mechanisms underlying boron-induced stress, including, antioxidative defense responses, lipid peroxidation, photosynthetic performance and B translocation-related gene expression measurements in order to determine the highest possible concentration for enhancing stress resilience during early development of sugar beet.

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P-97

The influence of decreasing iron fertilization on the photosynthetic activity, stress responses, and overall production of mandarin trees

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Keywords: chlorophyll fluorescence, excess iron nutrition, lipid peroxidation

Iron (Fe) plays a crucial role as a micronutrient for plants, contributing significantly to their metabolic processes. The majority of cell Fe, around 80-90%, is found in chloroplasts, thus any disruption in the foliar Fe balance has detrimental effects on chlorophyll biosynthesis and on the photosynthetic machinery [1]. To deal with iron deficiency, particularly in calcareous soils, iron is incorporated into fertilizers. However, excessive iron contents in soils can produce toxicity, especially in flooded soils that favor the presence of reduced, soluble Fe(II), and can lead to phytotoxicity by the over-production of reactive oxygen species [2]. The objective of this work was to reduce Fe chelate input 25% and 50% in a commercial mandarin orchard, in the presence and absence of ammonium sulphate. To evaluate the effect of the proposed managements, different physiological and biochemical parameters in the trees were analyzed, as well as the production and quality of the fruits. Field trials were conducted in a commercial orchard located in

Pozo Estrecho (Cartagena, Spain). Four years old mandarin (*Citrus reticulata*) trees cv. Nadorcott, grafted on '*Citrus Macrophylla*' and cultivated (planting frame of 417 trees/hectare) in a clay soil (pH 8-8.2) characterized by a low presence of organic C and a high amount of $CaCO_3$ were studied. During the trial, three different samplings were performed: short-, medium- and long-term (4 months, 9 months and 17 months after the start of treatments, respectively). The reduction in Fe fertilization produced a significant decline in Fe accumulation in soils, even at short-term, and interestingly leaf Fe levels did not show significant changes among different managements and at different sampling times. The lack of changes in leaf Fe contents correlated with the absence of differences in the chlorophylls and flavonols levels as well as in the photosynthesis performance, monitored by chlorophyll a fluorescence measurements. In general, the reduction in Fe did not increase the lipid peroxidation levels, an oxidative stress marker, and even lower values were observed at short- and medium-terms. Peroxidase (EC 1.11.1.7) activity, recognized as a biochemical indicator of Fe nutrition in plants, exhibited minimal variations in response to the agronomic management regarding Fe supply. Finally, both mandarin production and quality, measured in two different years, were not negatively affected by the reduction in Fe fertilization. Overall, our results support the feasibility of reducing Fe application without negatively affecting either the physiology of mandarin trees or their production, contributing to the decrease of soil and aquifers contamination.

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P-98

Effect of nitrogen application rates on agronomic efficiency and yield of rice: A meta-analysis

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Keywords: rice, *Oryza sativa*, agronomic efficiency(AE), grain yield, meta-analysis

Rice, a staple food crop for over half of the world's population, faces increasing demand, which is projected to rise by 44% by 2050. Despite a variety of concerns including greenhouse gas emission and groundwater contamination, farmers constantly apply chemical fertilizer to enhance rice yield. However, it is well known that only about 30-40% of applied nitrogen is typically utilized by crop plants. Of NUE indices, agronomic efficiency (AE) evaluates how much nitrogen fertilization contribute to the increase in grain yield. Therefore, this study was performed to investigate the effect of nitrogen application rates on an AE and grain yield of rice.

A systematic review was conducted using the Web of Science (WOS) database with the keywords 'Rice', 'N application', 'AE', and 'Main rice production country'. Field experiments, non-rice studies, and papers lacking AE or grain yield data were excluded, resulting in 24 selected studies. Hedges'd effect size was calculated for both AE and grain yield. Forest plots were generated to visualize the meta-analysis, and publication bias was assessed using funnel plots and Egger's regression test. MetaWin 3 (Rosenberg, 2024) software was used for meta-analysis, while subgroup analysis and graphing were performed with R language 4.3.2 and R Studio.

Both AE and grain yield were greatly dependent upon nitrogen application rates. The mean effect size for AE was 3.67 (95% CI: 2.99–4.35), and for grain yield it was 2.99 (95% CI: 2.42–3.57). Heterogeneity indices (I^2) were moderate, at 34.1% for AE and 37.8% for grain yield. Funnel plots and Egger's regression analysis indicated no significant publication bias. Subgroup analysis showed that AE peaked at a nitrogen application rate of 100–149 kg ha^{-1}, by contrast, grain yield tended to increase up to 250–299 kg ha^{-1} of N application. Among countries, India exhibited the highest AE, while China achieved the highest grain yield. Varietal subgroup analysis indicated that Indica types had higher AE, whereas Hybrid varieties showed greater yields. Overall, this study suggests that nitrogen fertilization strategies should be optimized based on application rate, varietal type, and regional agricultural practices to enhance rice productivity with reducing environmental risks.

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P-99

Optimzing selenium fertilization in *Medicago sativa* L.: Impacts on plant growth, Se uptake, and SeMet formation

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Keywords: agronomic biofortification, selenomethionine, red elemental Se, Se speciation

Agronomic biofortification with selenium (Se) is gaining recognition as an alternative to inorganic Se supplementation in animal nutrition, while also contributing to the production of Se-enriched crops for human consumption. Alfalfa (*Medicago sativa* L.), a widely used forage crop, shows potential for Se enrichment; however, the efficiency depends on both Se concentration and chemical form, especially in multi-harvest systems.

In this study, a greenhouse pot experiment was conducted to evaluate the effects of two ionic Se forms (sodium selenate and selenite) and red elemental selenium (Se^0) at concentrations of 1, 10, and 50 mg kg^{-1} (for ionic forms) and 10 and 50 mg kg^{-1} (for Se^0). Se treatments were mixed into the soil prior to sowing. Plants were harvested four times to monitor morphological, physiological, and biochemical responses, along with Se uptake dynamics.

Shoot length peaked at the third harvest (41–58 cm), with the longest shoots recorded under 10 mg kg^{-1} selenite. The average leaf-to-stem ratio was 56.3%, highest (67.5%) under 10 mg kg^{-1} red Se^0 at the fourth harvest. Leaf dry matter ranged from 25.8–30.7%. The 50 mg kg^{-1} selenate treatment was lethal to plants.

Across all treatments, leaves accumulated more Se than stems. Se content increased with dose, with selenate promoting the highest accumulation, followed by selenite and red Se^0. The highest Se level (586 mg kg^{-1}) was measured in plants treated with 50 mg kg^{-1} selenite. Selenate and selenite treatments showed peak uptake at the second harvest, declining thereafter. In contrast, Se^0 led to sustained Se accumulation, with significant increases observed in leaves at the third and fourth harvests under 10 mg kg^{-1}.

SAX HPLC-ICP-MS analysis revealed selenomethionine (SeMet) as the dominant organic Se compound and selenate as the main inorganic form. SeMet was more abundant in enzyme-extracted samples, indicating its potential bioavailability.

These results highlight the importance of Se form and dose in optimizing alfalfa biofortification strategies for both animal and human nutrition.

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P-100

Mechanisms of Zn- and Fe-tropism

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Keywords: tropism, zinc, iron, X-ray spectroscopy

Zinc and iron are crucial elements for all living organisms. Their deficiency is a global issue, highlighting the urgent need to explore diverse approaches for Zn and Fe biofortification in plants, which serve as the primary source of these essential nutrients. The heterogeneous distribution of these trace elements in soil complicates their uptake and distribution by plants. Although many studies have highlighted the phenomenon of Zn-tropism and Fe-tropism in plants, the mechanisms underlying this process remain unclear. Our project aims to uncover how plants determine the direction of growth in soils with uneven Zn and Fe (separately) distribution and how they subsequently distribute Zn/Fe within their organs.

Our research focuses on *Nicotiana tabacum* L. as a model plant. We cultivate the plants under Zn-deficient or Fe-deficient conditions using agar medium (¼ Knop solution with no added Zn or Fe). Once the root systems reach the desired size, we introduce a localized Zn source (¼ Knop agar medium supplemented with 20 µM Zn) or Fe source (¼ Knop agar medium supplemented with 400 µM Fe) and document the resulting growth patterns. Moreover, we employed X-ray spectroscopy to monitor the kinetics of Zn localization and quantify its concentration within the root system.

Our results showed that approximately 60% of plants grown under Zn-deficient conditions alter root growth direction toward the Zn source. Similar results obtained also for the Fe-tropism experiment. These findings suggest that specific root zones are responsible for sensing and uptaking Zn/Fe, distinct from root regions that do not contact the Zn/Fe source. Moreover, our results indicate that different mechanisms are responsible for Zn and Fe tropism. A deeper

understanding of tropisms could enhance Zn/Fe uptake efficiency in plants. This research may contribute to improving food quality by increasing the amount of Zn/Fe in edible parts and increasing fertilization efficiency.

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P-101

Analysis of key agro-morphological traits and nutritional components of foxtail millet germplasms

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Keywords: Foxtail millet, crude protein contents, total dietary fiber content, germplasm

Foxtail millet (*Setaria italica*) is a high-yielding crop with a short growth period. As a C4 plant, it is well-suited for cultivation in high-temperature environments. Due to these characteristics, foxtail millet is considered a promising candidate crop for addressing global warming and food security challenges. Additionally, it is nutritionally superior due to its high protein and vitamin B contents, providing functional benefits such as the prevention of hyperglycemia and hypertension. In South Korea, the increasing preference for multigrain rice over polished white rice, driven by the growing wellness trend, has enhanced the economic potential of foxtail millet. However, no local germplasms with improved nutritional properties have been developed to date.

The National Agrobiodiversity Center (NAC), Rural Development Administration (RDA), serves as South Korea's first internationally recognized World Seed Vault and Genebank by FAO-GCDT. NAC is committed to preventing the loss of crop germplasms and promoting their sustainable utilization. The center preserves over 280,000 plant germplasms, including more than 2,000 foxtail millet germplasms. However, research on their nutritional components remains insufficient.

To identify potential breeding materials with enhanced nutritional properties, we analyzed the crude protein and total dietary fiber contents of 150 foxtail millet germplasms preserved at NAC. The results showed that crude protein content ranged from 6.851% to 14.709%, with four germplasms exhibiting protein levels exceeding 14.0%: IT137723 (SI46, Thailand, 14.709%), IT153488 (ISE1206, Russia, 14.147%), IT153502 (ISE1247, Russia, 14.084%), and IT137732 (SI55, Russia, 14.001%).Total dietary fiber content ranged from 9.813% to 22.894%, with one germplasm exceeding 22.0%: IT137691 (SI13, Thailand, 22.894%). Among the analyzed germplasms, IT137691, though non-erect, exhibited the thickest stem (9.5 mm), while IT153488, with a slightly thinner stem (9.0 mm), displayed an erect growth habit. These two germplasms demonstrated high nutritional value and superior lodging resistance, making them promising candidates for improved cultivation efficiency.

This study provides fundamental data for selecting high-nutrient foxtail millet breeding materials. The findings could also assist plant breeders and contribute to reducing the time and effort required for the development of improved varieties.

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PHOTOSYNTHESIS AND LIGHT REGULATION

P-102

The involvement of components of the signalling pathway in the adaptation of *Arabidopsis thaliana* plants to increased doses of UV-B radiation

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Keywords: UV-B radiation, *Arabidopsis thaliana*, photomorphogenesis, stress tolerance, antioxidants, non-photochemical quenching (NPQ)

Ultraviolet-B radiation (UV-B, 280–320 nm) plays a dual role in plant physiology: it induces oxidative stress and damages DNA while simultaneously activating defense mechanisms by regulating secondary metabolism and photosynthetic activity. In recent years, UV-B has been actively used in biotechnology to stimulate the synthesis of biologically active compounds, such as flavonoids and anthocyanins, which possess antioxidant and antibacterial properties. However, the mechanisms coordinating light signalling between UV RESISTANCE LOCUS 8 (*UVR8*) and other photoreceptors remain insufficiently understood.

This study revealed a novel level of regulation in the *UVR8* signalling pathway associated with the proteins BLUE LIGHT INHIBITOR OF CRYPTOCHROMES 1,2 (*BIC1,2*). *BIC1,2* not only inhibits cryptochromes but also modulates *UVR8*-dependent defense mechanisms, preventing the hyperactivation of *UVR8* under UV-B irradiation. In *bic1,2* mutants, an enhanced UVR8-dependent response was observed, including hyperexpression of antioxidant defense genes (*APX, GPX*) and increased flavonoid biosynthesis. This increase was accompanied by an increase in non-photochemical quenching (NPQ), which prevented damage to PSII.

Additionally, it has been established for the first time that SUPPRESSOR OF PHYA-105 (*SPA*) influences not only the stability of *HY5* but also the water balance of plants under UV-B stress. In spa mutants, despite the accumulation of flavonoids, disruptions in stomatal regulation and a reduction in stomatal conductance were observed, impairing stress adaptation.

HY5 is a key regulator of the UV-B response that is activated by *UVR8* and induces the expression of CHALCONE SYNTHASE (*CHS*) for flavonoid synthesis. However, experiments have shown that in the absence of *HY5*, plants lose the ability to mount an effective photoprotective response: in hy5 mutants, the activation of NPQ was reduced, leading to increased photodamage to photosystem II.

A novel functional relationship between *UVR8, BIC1,2, HY5,* and *SPA* has been identified, ensuring a balance between photoprotective mechanisms and the maintenance of photosynthetic activity. These results open new prospects for the biotechnological modification of crops, enhancing their resilience to UV-B under changing climatic conditions.

This work was supported by the grant from the Russian Science Foundation (project no. 23-14-00266).

P-103

Knocking out doubts: How to resolve the role of Lhcb2 in plant photoprotection

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Keywords: photosynthesis, photoprotection, PSII

The major light-harvesting complex of photosystem II (LHCII) is the most abundant pigment-protein complex on Earth, accounting for approximately 70% of the pigments associated with PSII. LHCII enhances the absorption cross-section of the supercomplex and plays a crucial role in light harvesting regulation. It is composed of the Lhcb1-3 gene products, expressed with a stoichiometry of 8 (Lhcb1): 3 (Lhcb2): 1 (Lhcb3) that is conserved among angiosperms. LHCII is essential for non-photochemical quenching (NPQ), which dissipates excess excitation energy as heat, thereby preventing photoinhibition. It has been proposed that among the LHCII isoforms, Lhcb1 is fully competent to engage in NPQ, while the contributions of Lhcb2 and Lhcb3 appear negligible. Since Lhcb1 is the most abundant LHCII isoform, impaired NPQ in plants devoid of Lhcb1 may indirectly stem from an uneven distribution of the quenching sites within the pigment bed. By employing *Arabidopsis* lines missing specific antenna components, namely Lhcb1, Lhcb2 or the entire trimeric LHCII, we investigated their role in catalyzing NPQ. In the *koLhcb1* line, total LHCII level decreased by 70% compared to the WT; however, the maximal NPQ was only slightly affected (-20%); moreover, the relative abundance of PSII

supercomplexes remained consistent across WT, *koLhcb1* and *koLhcb2* plants. To simplify the relation between LHCII composition and amplitude of quenching, we examined the dependence of NPQ response by expressing various levels of Lhcb1 or Lhcb2 as the sole LHCII components. We found that maximal NPQ increased with complementation by Lhcb1 at different levels, up to saturation at a LHCII content similar to WT plants. Contrary to previous reports, the expression of Lhcb2 as the sole LHCII isoform exhibited a dependence of maximal NPQ on LHCII content that closely matched that of lines expressing Lhcb1 only. Our results reveal that both Lhcb1 and Lhcb2 subunits contribute equally to the quenching response, implying that Lhcb2 is fully competent for NPQ activity.

P-104

Photosynthetic adaptations of *Galdieria phlegrea* under autotrophic and heterotrophic condition

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Keywords: *Galdieria phlegrea*, photosynthetic apparatus, autotrophic and heterotrophic growth

Galdieria phlegrea (strain 734) is a unicellular, polyextremophilic red alga of the class *Cyanidiophyceae* adapted to colonize highly acidic and thermal environments [1]. It exhibits metabolic flexibility, capable of growth under both autotrophic and heterotrophic conditions, utilizing different organic carbon sources as substrates [1].

This study investigated the physiological and structural differences of its photosynthetic apparatus when grown under autotrophic and heterotrophic conditions. To this aim, we performed fluorescence measurements and quantified chlorophyll a (Chl a) content.

Our results revealed significant changes in the photosynthetic apparatus depending on the growth mode, providing new insights into the metabolic flexibility of *G. phlegrea*.

Specifically, the maximum quantum efficiency of PSII (Fv/Fm) was significantly lower under heterotrophic growth compared to autotrophy, indicating a reduced photochemical efficiency likely due to the downregulation of the photosynthetic apparatus when photosynthesis is not essential for energy production. Consistently, the effective quantum yield of PSII (Y(II)) was also higher in autotrophic cells, reflecting a greater proportion of open, functional PSII reaction centers when photosynthesis is the primary metabolic pathway.

The chlorophyll a content was markedly higher under autotrophic conditions, as expected, because light-harvesting complexes are more abundant when cells rely on photosynthesis for growth and energy.

Interestingly, measurements of maximum transient absorption signal of PSI under actinic light (Pm') and the effective quantum yield of PSI (Y(I)) showed that, although the amount of PSI was lower under heterotrophic conditions, the existing PSI complexes remained fully active and functional, suggesting a selective retention of operational photosystems even when photosynthesis is downregulated.

Understanding these adaptations could contribute to optimizing the biotechnological exploitation of *G. phlegrea*.

[1] Salbitani, G., Cipolletta, S., Vona, V. et al. Heterotrophic Cultures of Galdieria phlegrea Shift to Autotrophy in the Presence or Absence of Glycerol. J Plant Growth Regul 40, 371–378 (2021). https://doi.org/10.1007/s00344-020-10109-0

P-105

Beyond protection: Anatomical adaptations, plastid dynamics, and photosynthetic potential in horse chestnut (*Aesculus hippocastanum* L.) bud scales

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Keywords: bud scale photomorphogenesis, etiolation plasticity, low light adaptation, photosynthesis, plastid differentiation

Bud scales are primarily known for their protective role in shielding dormant shoot apices from environmental stress, but their potential physiological functions remain poorly understood. This study investigates the anatomical features and physiological adaptations of bud scales in horse chestnut (*Aesculus hippocastanum* L.), with particular emphasis on plastid differentiation and their capacity for photosynthetic activity. Using transmission electron microscopy, we documented dynamic plastid transformations in bud scales across

seasons, including the presence of etio-chloroplasts in low-light conditions and their conversion to amylo-chloroplasts during winter dormancy.

Photosynthetic parameters were quantified through chlorophyll-*a* fluorescence measurements and PAM fluorometry, revealing detectable photosynthetic activity despite extreme light limitation. Transmission spectroscopy confirmed that less than 1% of incident light penetrates through the bud scale layers, creating a unique microenvironment for plastid development. Our findings demonstrate that bud scale plastids maintain photosynthetic machinery and undergo light-dependent transformations, suggesting their potential role in energy metabolism during dormancy.

These results challenge the conventional view of bud scales as merely passive protective structures and instead position them as active contributors to plant physiology through their photosynthetically competent plastids. The study provides new insights into the remarkable adaptations of overwintering structures in perennial plants.

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P-106

Effect of long-term red light inter-lighting on carbohydrate metabolism and morphological traits of tomato plants

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Keywords: red light; inter-lighting; carbohydrate metabolism

The tomato, *Solanum lycopersicum* (L.), is one of the most sought-after and most widely grown greenhouse crops worldwide. In order to achieve more efficient production, a lot of research has been conducted to determine which of the many influencing factors can be changed to increase crop yield and quality in the most economical and environmentally friendly way possible. One of the most important such parameters is light. Light itself plays a prominent role in the entire life cycle of plants, as it affects everything from germination to plant development, reproduction and defense against pathogens. Several experiments have been conducted on the effects of light at different times of the day with different wavelengths and different time intervals, on plants. Red light, which we also used in our research, has been shown to have a beneficial effect on photosynthetic efficiency, biomass, and the enhancement of defense processes against various biotic stressors. In our experimental setup, we used so-called "inter-lighting". In this system, plants receive supplemental lighting between the leaf layers. As a result, the light is better distributed and can be utilized more evenly, even on multiple leaf layers. In our research, we compared two tomato plants of different genotypes, one was *Money Maker* and the other was *Never ripe*, a variant with a mutation in its ethylene receptor. We were curious to see what different „inter-lighting" conditions could affect plants in an ethylene-dependent manner. We measured the photosynthetic activity, CO_2 assimilation, carbohydrate metabolism and plant morphology changes.

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P-107

Effect of salt stress on plastid ultrastructure and function in eight *Arabidopsis* genotypes lacking various thylakoid ion channels and transporters

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Keywords: chloroplast, etioplast, salt stress, thylakoid, CLCe KEA3, VCCN, *Arabidopsis*

Increasing soil salinity is becoming a major global challenge, significantly impairing agricultural productivity and posing a serious threat to food security. A deeper understanding of how it affects plant metabolism, especially photosynthetic activity, might help the breeding of salt tolerant crops or find ways to enhance the salt stress tolerance of crops. Salt stress impacts crops in different ways, disrupting the structural integrity and functional capacity of plastids. In this work, plastid ultrastructure was compared in the cotyledons or leaves of dark- or light-grown thale cress (*Arabidopsis*

thaliana L.) plants of different developmental stages under control conditions and after the influence of short-term (30 min; 200 or 300 mM NaCl) or long-term salt stress treatments (4 h, 600 mM NaCl:KCl, 1:1). In addition to the wild-type (Columbia-0) plants, we had single, double, and triple mutants of the thylakoid-located CLCe chloride ion channel, KEA3 potassium$^+$/H$^+$ antiporter and VCCN1 voltage-gated chloride ion channel, which did not contain these components. Based on our transmission electron microscopic analyses, we can conclude that salt stress does not affect the structure of the photosynthetic apparatus of mature chloroplasts in old leaves (4- and 8-week-old). However, in the etioplasts of the cotyledons of dark-grown triple mutant seedlings salt stress induced the formation of vesicles, and in the cotyledon chloroplasts of young light-grown seedlings it also caused the swelling of the lumen of the stroma thylakoids. In the young chloroplasts of the cotyledons, membrane reorganization processes indicating selective chloroplast autophagy in both wild-type and triple mutant plants started already after 4 hours of salt shock (600 mM NaCl:KCl) treatment. Photosynthetic activity (especially Qy light values) was significantly decreased in several mutants. Looking at the OJIP curves, in all cases where the CLCe channel was missing in point I, because of the treatment, higher chlorophyll fluorescence value was measured than the untreated sample case. Our results confirmed the sensitivity of etioplasts and young chloroplasts to salt stress, and also outlined the complex role of the various thylakoid ion transport components in the preservation of the structural and functional stability of the photosynthetic apparatus and thylakoid membranes under salt stress.

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P-108

Regulation and topology of the plastidial K+/H+ antiporter ATKEA2

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Keywords: K+/H+ antiporter, Chloroplast, Arabidopsis

We have shown previously that the Arabidopsis plastid envelope K$^+$/H$^+$ antiporters KEA1 and KEA2 play an important role in stromal pH regulation, especially in light to dark transitions. Double mutant disruption mutants that lack both proteins have a considerably higher stromal pH compared to wild type plants, while cytosol pH and membrane potential are lower. Chloroplast, leaf and plant development is seriously compromised in these plants. This is most likely caused by the altered pH or ionic conditions in the stroma, which affects plastid and nuclear gene expression by retrograde signaling. Regulation of gene expression by modulation of KEA1 and KEA2 activity by light could be an important mechanism for signaling of the chloroplast energy status to the cell. In plants, envelope KEA1 and KEA2 as well as the thylakoid membrane KEA3 protein contain a C-terminal KTN (K$^+$ transport/nucleotide-binding) also called RCK (regulator of K$^+$ conductance) domain. It was recently shown that the KTN domain of KEA3 can bind ATP and NADPH, which is most likely providing a mechanism for regulation by light. We now show that the K$^+$/H$^+$ antiport activity of KEA2 is regulated by ATP and NADPH, which depends on the presence of the KTN domain.

Plant KEA proteins show high homology with the bacterial transporter KefC. The plant KEA1 and KEA2 proteins have acquired an additional 550 amino acid long "Soluble" N-terminal domain of unknown function. The structure of KefC was recently resolved, showing it contains 13 Trans Membrane Helices. For KEA1 and KEA2 it was shown that both N-and C-terminus reside inside the stroma, meaning that it should at least contain an even number of TM helices. We were able to show that the N-terminal domain of KEA2 indeed contains one trans membrane helix by expression of deletion mutants in yeast. KEA2 thus contains a total of 14 trans membrane helices, which is correctly modelled by the Alpha-fold prediction server.

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P-109

Involvement of ethylene signaling cascade in dehydration stress response

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Keywords: ethylene signaling, dehydration stress, *Arabidopsis thaliana*

Ethylene is one of the main phytohormones that regulate a number of developmental processes in plant life cycle. It also participates in the control of adaptive reactions towards adverse factors of the environment. The gaseous hormone actively interplays with ABA and auxines in the regulation of response to dehydration.

Comparative functional analyses of signaling mutants have been employed to elucidate the major elements of the signaling cascade that are affected by dehydration. The experiments were conducted with *A. thaliana* wild type (Col-0) and mutant plants: the constitutive ethylene response mutant (*ctr1-1*) and an insensitive to ethylene mutant (*ein2-1*), grown on ½ MS media. Sorbitol (75 mM) was used as dehydration stress inducing substance. We analysed the gene expression levels of major ethylene signaling cascade player's *(ETR1, CTR1, EIN2, EIN3, EBF1, EBF2*) by Real Time qPCR analysis during the early stress response (72 h) and after prolonged exposure to dehydration (21 Days). *EBS::GUS* staining activity during the stress period was monitored to estimate organ-specific dynamic changes in the reporter as a proxy of ethylene signaling activity.

The obtained results displayed dynamic changes of transcripts coding for elements from the canonical ethylene signaling pathway. That was accompanied by consistently decreased ethylene signaling in roots and shoots during the dehydration stress reported by the *EBS::GUS* construct. During the early stage of stress (at 72 h), both the wild type and the mutant plants exhibited mostly decreased expression of the monitored genes. The wild type plants showed similar trend of gene expression levels after prolonged dehydration. The constitutive mutant *ctr1-1* exhibited a strong inhibition of the transcripts coding for EIN3, the ethylene master transcription factor, at the later stages of the stress. This was accompanied by relatively higher expression of the genes coding for the F-box proteins EBF1 and EBF2 that target EIN3 for degradation in the proteosome complex. The transcript profiling of the studied genes in *ctr1-1* suggest intensified turnover of EIN3 ethylene transcription factor after prolonged exposure to sorbitol that could be linked to the better survival of the mutant upon dehydration.

P-110

Exogenous brassinosteroids enhance *Arabidopsis thaliana* immunity and modulate phytohormonal crosstalk: Insights from comparative research

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Keywords: brassinosteroids, plant biotic stress resistance, phytohormonal crosstalk, *Arabidopsis thaliana*

Brassinosteroids (BRs) are known as important regulators of plant growth, development, and defense mechanisms. This study aimed at disclosing the effects of exogenous application on 24-epibrassinolide (EBL) and brassinazole (Brz), a BR biosynthesis inhibitor, on *Arabidopsis thaliana* resistance to *Pseudomonas syringae* infection. We have also evaluated the effect of EBL on endogenous phytohormone levels. Using flood inoculation and syringe infiltration methods, we found that EBL significantly increased plant resistance to pathogen infection. On the other hand, Brz treatment led to increased susceptibility, but this effect was partially mitigated by co-application with EBL. Foliar application of EBL was more effective than media supplementation in terms of resistance enhancement.

To further understand underlying BR-mediated defense and growth-regulating mechanisms, we analyzed endogenous phytohormone levels using high-performance liquid chromatography-mass spectrometry (HPLC-MS). EBL application led to increased dihydrophaseic acid (DPA) levels without significantly affecting abscisic acid (ABA) or phaseic acid (PA) concentrations. Additionally, EBL reduced salicylic acid (SA) levels, indicating an antagonistic interaction with SA-dependent defense pathways. Speaking of growth-stimulating phytohormones, auxin metabolism was also influenced: indoleacetic acid (IAA) and IAA-glucose ester

contents were significantly elevated. Furthermore, EBL treatment has changed cytokinin profiles, increasing isopentenyl adenine-7-glucoside (iP7G) and cis-zeatin riboside monophosphate (cZRMP).

These findings are consistent with our previous research on soybean (*Glycine max*), which demonstrated that foliar application of 24-epicastasterone (ECS), a member of brassinosteroids family, caused an increase in endogenous IAA while reducing SA, JA, and ABA levels. The consistency of BR-mediated hormonal modulation across different plant species suggests their potential application in improving crop resistance to biotic stress and optimizing growth under varying environmental conditions.

This work was supported by the EURIZON H2020 (EURIZON FELLOWSHIP PROGRAMME Remote Research Grants, grant #EU-3042-137) and Scholarship of the President of Ukraine for Young Scientists in 2023-2024.

P-111

Comprehensive profiling of phytohormones and polyamines: A novel LC-MS/MS approach for plant metabolic studies

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Keywords: ethylene, polyamines, phytohormones, LC-MS/MS

The plant hormone ethylene and polyamines play pivotal roles in plant development and stress responses, with their metabolic pathways being tightly interconnected by the adjacent Yang cycle. Despite the compounds' importance, their quantification remains a significant analytical challenge, partially due to their low retention on reversed-phase columns, with verified profiling methods lacking.

Here, we present a novel and validated liquid chromatography–tandem mass spectrometry (LC-MS/MS) based approach, that enables the profiling of a broad spectrum of phytohormones, ethylene precursors, polyamines, and related metabolites from a single biological sample. This integrated approach allows determination of 15 phytohormonal compounds, including auxins, cytokinins, jasmonic acid, abscisic acid, salicylic acid, and 1-aminocyclopropane-1-carboxylic acid, alongside 14 key metabolites from polyamine and amino acid pathways, such as putrescine, spermidine, spermine, thermospermine, L-arginine, L-citrulline, and L-ornithine.

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

By integrating the quantification of these groups of plant growth regulating compounds, this novel methodology provides a comprehensive tool for elucidating complex metabolic interactions. It enables indirect ethylene studies while simultaneously exploring the interconnected roles of polyamines and phytohormones. This advancement holds substantial promise for enhancing our understanding of plant metabolic adaptability and regulatory mechanisms under diverse environmental conditions.

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P-112

Understanding changes in aroma metabolism during development and ripening of strawberry fruit, through exogenous hormonal treatments

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Keywords: Abscisic acid (ABA), alcohol acyltransferase, ester biosynthesis, strawberry fruits, fruit ripening process

Fragaria x ananassa, commonly known as strawberries, are highlighted for their exceptional flavor, a result of the

harmonious blend of acids, sugars, and volatile compounds. Among these, aroma stands out as a crucial factor influencing consumer preference. Key enzymes such as pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) are pivotal in ethanol production during fermentation. PDC initiates the conversion of pyruvate into acetaldehyde and CO_2, while ADH facilitates the subsequent transformation of acetaldehyde into ethanol. These enzymatic processes are crucial in ethanol metabolism, acting as precursors for the formation of esters and other aromatic compounds that lend strawberries their distinctive aroma. Additionally, Alcohol Acyltransferase enzymes (AATs) play a crucial role in catalyzing acyl group transfer, significantly contributing to the diversity of fruit aromas, including strawberries. This study explores the impact of the effects of Abscisic acid (ABA) hormonal treatment on the expression of *FaPDC*, *FaADH*, and *FaAAT* enzyme-coding genes, as well as their correlation with volatile ester production in strawberries. The treated fruits exhibited substantial upregulation of three *FaAAT*, *FaADH*, and *FaPDC* genes compared to the control group. This heightened gene expression directly correlated with increased volatile ester production, key contributors to strawberries aroma and flavor profile. These findings underscore the crucial role of ABA hormone in modulating the metabolic pathway of volatile esters by positively regulating *FaPDC*, *FaADH*, and *FaAAT* genes. Beyond elucidating the molecular mechanisms underlying aromatic compound biosynthesis in fruits, this study highlights the potential of hormonal treatment as a valuable tool for enhancing the sensory attributes of agricultural products.

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P-113

Ethylene biosynthesis is regulated by treatment with GR24 in etiolated rice seedlings

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Keywords: ACC oxidase, ACC synthase, ethylene biosynthesis, SAM synthetase, strigolactone

In plants, developmental or environmental stresses activate a suite of different phytohormones that trigger biochemical and/or morphological adaptations. The gaseous phytohormone ethylene has a major effect on the plant life cycle from germination onward. Ethylene biosynthesis is tightly regulated by external and internal cues. In etiolated seedlings of Arabidopsis and rice, various phytohormones affect ethylene biosynthesis through transcriptional and/ or post-transcriptional regulation of 1-aminocyclopropane-1-carboxylic acid (ACC), ACC synthases (ACS), and ACC oxidases (ACO). This study showed strigolactone also affected ethylene biosynthesis in dark-grown rice seedlings. Str igolactone treatment altered levels of S-ADENOSYLMETHIONINE SYNTHASES (OsSAMSs) and ACC SYNTHASES (OsACSs) transcripts, which encode enzymes involved in the initial steps of ethylene biosynthesis. The application of strigolactone reduced ethylene production, however, by decreasing transcription of OsACO genes, thus negatively affecting the final step of ethylene biosynthesis. In addition, treatment with strigolactone resulted in a phenotype in which the coleoptiles of dark-grown rice seedlings were shortened, contrary to treatment with ACC. These results reveal the tight correlation between strigolactone and ethylene biosynthesis.

P-114

Comparative analysis of dehydrin expression in ethylene signaling mutants *ctr1-1* and *ein2-1*

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Keywords: *Arabidopsis thaliana*, dehydrins, ethylene signaling mutants

Dehydrins (DHNs) belong to a family of stress-inducible proteins with protective functions. They accumulate in particularly high concentrations during the late stages of ontogenesis, performing an important physiological role associated with seed maturation. Their synthesis is triggered by various adverse environmental factors, such as drought, frost, salinity, and extreme temperatures. Due to their unique molecular properties, DHNs stabilize the cell plasma membrane and maintain the native conformation of macromolecules, ensuring the proper

functioning of enzymes, structural proteins, and nucleic acids. They are characterized by high hydrophilicity, thermostability, and a low degree of secondary and tertiary structure. One of the main stress hormones, abscisic acid (ABA), is known to participate in the control of dehydrin expression. However, the involvement of other signaling pathways, particularly ethylene, in the regulation of this class of proteins remains insufficiently explored. We tested the effect of the ethylene precursor ACC on the expression of six annotated *A. thaliana* genes in wild type (Col-0) and ethylene insensitive (*ein2-1*) and constitutive (*ctr1-1*) mutants. The increased levels of *XERO1* and *ERD14* transcripts in *ctr1-1* mutant and their relatively low abundance in the ethylene-insensitive *ein2-1* seedlings when the ethylene precursor ACC was present in the nutrient media suggest that ethylene exerts control over the expression of these DHN genes. *HIRD11* (coding for a KS-type dehydrin) did not exhibit any significant differences in its transcript profiles among the tested genotypes, suggesting that ethylene is less involved in the regulation of its expression. Further, we performed comparative analyses of the growth parameters, stress markers (L-proline, malonedialdehyde and hidrogen peroxide) in plants from the three genotypes grown grown on ½ MS medium -/+ 75 mM sorbitol. The mutant *ctr1-1* exhibited the best growth parameters under dehydration stress that corroborate well with the relatively lower levels of oxidative stress markers measured in its tissues. We also performed comparative gene expression and immunodetection analyses of dehydrins in plants from the three genotypes under dehydration. *DHN* transcript profiling revealed that ethylene signaling plays a central role in controlling the expression of *XERO1*, *LEA14* and *RAB18*, and that the gaseous hormone is not of primary importance for the regulation of *HIRD11* and *XERO2*. The immunoblot analyses of Col-0, *ctr1-1* and *ein2-1* seedlings subjected to prolonged dehydration also showed that ethylene suppresses the accumulation of low molecular weight Y-segment containing dehydrin with an approximate MW of 20 kDa.

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P-115

Comparative metabolite profiling approach reveals the complexity of auxin metabolism across plant species

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Auxin is a central regulator of plant growth and development, with its metabolism and homeostasis tightly controlled to ensure precise spatial and temporal regulation of signaling. While extensive research has elucidated key auxin biosynthesis, transport, and degradation pathways, recent discoveries suggest that auxin metabolism is far more complex and evolutionarily diverse than previously thought. The widely studied indole-3acetic acid (IAA) inactivation pathway in Arabidopsis involves multiple pathways, including conjugation with amino acids and sugars, as well as oxidative degradation to produce inactive auxin metabolites.

We developed a novel approach that combines micro-scale extraction with ultra-sensitive liquid chromatography-mass spectrometry, enabling the profiling of a broad range of auxin metabolites across various plant models. Using this method, we conducted a comprehensive analysis of auxin metabolites in *Arabidopsis thaliana* and crop species, identifying new endogenous auxin metabolites. Our findings revealed that metabolite profiles varied significantly between species and among different seedling tissues. Moreover, auxin metabolite levels were strongly influenced by the growth stage of the seedling, highlighting the crucial role of IAA metabolism in maintaining phytohormonal balance during plant development. Through an in-depth investigation of phenylacetic acid (PAA) metabolism, we demonstrated that both auxins, PAA and IAA, share a core metabolic framework, uncovering a complex regulatory network that governs auxin homeostasis. These insights advance our knowledge of auxin-specific metabolic networks and highlight the unique complexity within plant hormone regulation.

P-116

Dinucleoside polyphosphates: Emerging players in plant signaling networks

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Keywords: dinucleoside polyphosphates (NpnNs), plant stress signaling, purinoreceptors (P2K1/P2K2)

Dinucleoside polyphosphates (Np_nNs) are endogenous compounds that accumulate in cells under stress conditions, functioning as secondary messengers. In plants, the phenylpropanoid pathway produces a range of protective metabolites, including flavonoids, stilbenes, and lignin. Our previous work demonstrated that Np_nNs modulate this pathway in *Arabidopsis thaliana* and *Vitis vinifera*, supporting their role in plant stress responses. Specifically, among the purine- and pyrimidine-type Np_nNs analyzed, Ap_nA promoted stilbene synthesis, whereas Cp_nC suppressed this process but strongly induced the expression of lignin biosynthetic genes. Moreover, we recently observed that Ap_4A, one of the Np_nNs, is accumulated in response to cadmium stress in *Arabidopsis thaliana* seedlings.

Recent findings indicate that Ap_4A is recognized by the plasma membrane purinoreceptor P2K1/DORN1. To date, two plant purinoreceptors, P2K1 and P2K2, have been identified, both containing extracellular ATP-binding sites. Structural analysis revealed that the nucleotide-binding pockets of P2K1 and P2K2 are highly conserved. *In silico* docking studies further showed that Ap_4A and Cp_4C interact with the extracellular lectin domains of both receptors; however, Ap_4A exhibited significantly higher binding affinity than Cp_4C, suggesting a structural basis for differential ligand recognition.

The emerging evidence on the role of dinucleoside polyphosphates (Np_nNs) in modulating stress-related pathways in plants opens new avenues for future research. Our findings, demonstrating the influence of Np_nNs on the phenylpropanoid pathway and their interaction with purinoreceptors, suggest that these molecules could serve as crucial regulators of plant stress responses. Further studies are needed to fully elucidate the signaling networks downstream of Ap_4A–P2K1/P2K2 interactions, particularly in the context of different stress conditions and tissue types.

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P-117

Vascular system formation: An auxin-dependent physiological process involved in plant organogenesis

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Keywords: auxin; urea derivatives; vascular pattern

The vascular system is fundamental for land plants, ensuring the long-distance transport of water, nutrients, and signaling molecules. In *Arabidopsis thaliana*, vascular patterning is tightly regulated by the hormone auxin, which, according to the canalization hypothesis, promotes the formation of self-organizing transport channels via feedback regulation of its own polar transport. Vascular differentiation typically occurs where elevated auxin levels intersect with responsive cell populations.

It is thought that the small yet functionally active apoplastic fraction of Auxin Binding Protein 1 (ABP1) act in this extracellular acidic environment as a receptor mediating some auxin-driven processes such as canalization.

Here, we present preliminary data on the effects of two synthetic urea derivatives: *N, N'*-bis-(2,3-methylene-dioxyphenyl) urea (2,3-MDPU) and 1,3-di(benzo[d]oxazol-5-yl) urea (5-BDPU) on vascular development in cotyledons of *Arabidopsis thaliana*. Seedlings were grown *in vitro* under various concentrations of the compounds alone, in mixture with auxin or in single sequential culture of both types of compounds (urea derivative and auxin). When seedlings are cultured in the presence of the compounds alone the vascular pattern is very similar to that of hormone free (HF) condition.

In the simultaneous presence of auxin plus the urea derivatives, the results suggest that these molecules reduce auxin responsiveness and may alter vascular patterning.

By contrast, in the sequential treatments it seems that the last one is responsible for the result.

In addition, molecular docking studies indicate that both 2,3-MDPU and 5-BDPU can potentially bind to the ABP1 receptor in the absence of auxin, with distinct binding energies.

Furthermore, enzymatic assays show that these synthetic urea derivatives inhibit cytokinin oxidase / dehydrogenase (CKX) activity.

Future researches will include:

- a molecular expression analysis of some genes related with vascular differentiation and auxin transport (including the ones that behave as cytokinin-responsive genes).
- an analysis of the endogenous cytokinin content in the presence of the urea derivatives.

P-118

Strigolactones positively affect ripening of tomato berries

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Keywords: fruit, GR24, ABA, carotenoids

Strigolactones (SLs) play a role in several plant developmental processes through the interaction with other phytohormones. Nevertheless, there is a lack of information regarding their role during fruit ripening. It is known that in grapevine berries, exogenous SLs interact with abscisic acid-mediated accumulation of anthocyanins. In Arabidopsis, SLs enhance ethylene action in leaf senescence. Onset of ripening in tomato, a climacteric fruit, is caused by a peak of ethylene, which is linked to a previous increase in ABA content.

To investigate the effects of SLs on metabolic and physiological processes of tomato fruits during the post-harvest phase, we applied GR24, a synthetic SL analogue, to berries of the tomato genotypes Micro-Tom and Butalina. Berries were collected at the 'mature green' stage and divided into two homogenous groups, one injected with a solution of GR24 5 µM, and the other with a mock solution used as reference control. The treatment was effective in promoting a faster change of berry skin color from green to red, particularly evident in Butalina. GR24 treatment on Micro-Tom induced upregulation of genes involved in lycopene (*SlPDS* and *SlPSY1*) and in ethylene biosynthesis, such as *SlACO1* and *SlACS2*. In Butalina, GR24-treated berries showed up-regulation of the same genes within 72 hours after treatment, together with the activation of other genes involved in berry ripening (i.e. *SlCNR*) and in cell-wall degradation (i.e. *SlPL*). Quantification of secondary metabolites revealed an increase in phytoene and carotene isomers, as well as in ethylene production, and a progressive reduction of fruit firmness in response to GR24 treatment. ABA content and *SlNCED1* transcription were negatively affected by GR24 treatment, while ABA catabolism-related genes, *SlCYP707A1* and *SlUGT75C1*, were activated in both genotypes. To further support the findings obtained by treatment with exogenous SL, we monitored the maturation of genetically SL-depleted plants, silenced for the *SlCCD7* SL-biosynthetic gene in the M82 background (*ccd7*), compared to their WT. In agreement with the observations on GR24-treated berries, we observed a delay in ripening of the *ccd7* berries.

Our results show that exogenous and endogenous SLs positively affect tomato berry ripening. Since ABA levels were negatively affected by SL, we hypothesize that ABA sensitivity was increased, similarly as previously reported in tomato leaves.

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P-119

Spermine deficiency shifts the balance between jasmonic acid and salicylic acid-mediated defense responses in *Arabidopsis*

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Keywords: polyamines, coronatine, JA, SA, ER stress, galactolipids, *Pseudomonas syringae*, *Botrytis cinerea*

Polyamines are small aliphatic polycations present in all living organisms. In plants, the most abundant polyamines are putrescine (Put), spermidine (Spd) and spermine (Spm). Polyamine levels change in response to different pathogens, including *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000). However, the regulation of polyamine metabolism and their specific contributions to defense are not fully understood. Here we report that stimulation of Put biosynthesis by *Pst* DC3000 is dependent on coronatine (COR) perception and jasmonic acid (JA) signaling, independently of salicylic acid (SA). Conversely, lack of Spm in *spermine synthase* (*spms*) mutant stimulated galactolipids and JA biosynthesis, and JA signaling under basal conditions and during *Pst* DC3000 infection, while compromised SA-pathway activation and defense outputs through SA-JA antagonism. The dampening of SA responses correlated with COR and *Pst* DC3000-inducible deregulation of *ANAC019* expression and its key SA-metabolism gene targets. Spm deficiency also led to enhanced disease resistance to the necrotrophic fungal pathogen *Botrytis cinerea* and stimulated endoplasmic reticulum (ER) stress signaling in response to *Pst* DC3000. Overall, our findings provide evidence for the integration of polyamine metabolism in JA and SA-mediated defense responses, as well as the participation of Spm in buffering ER stress during defense.

P-120

Evaluation of host plant resistance to *Cnaphalocrocis medinalis* in rice

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Keywords: biotic stress, insect pest, *Oryza sativa*, physical defenses

The rice leaffolder (*Cnaphalocrocis medinalis*) is a major insect pest affecting rice cultivation in Asia and Australia. To reduce over-reliance on chemical pesticides as the primary method of pest control, it is essential to harness the natural resistance found in various rice varieties. Previous research has identified the rice varieties Balam and Gora (from Bangladesh) and Choorapaudy (from India) as highly resistant to leaffolder, although the underlying resistance mechanisms remain poorly understood. This study compared plant morphology and trichome characteristics of these varieties with those of the Taiwanese resistant variety Baiqiaowan and the susceptible variety TN1. Among the morphological traits, Baiqiaowan exhibited longer leaves, while Choorapaudy had narrower leaf blades-features that may interfere with the leaf-rolling behavior of leaffolders. Both Baiqiaowan and Choorapaudy also had longer and denser trichomes, which are likely to hinder larval movement on the leaf surface. Notably, leaffolders took more time to initiate leaf rolling on Baiqiaowan, a delay not observed with Choorapaudy. Ongoing research includes (1) the use of scanning electron microscopy to explore structural differences in trichomes between Baiqiaowan and Choorapaudy, and (2) a comparison of insect growth across all the studied resistant rice varieties.

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P-121

The potential of L-arginine-conjugated chitosan nanoparticles in the induction of Systemic Acquired Resistance (SAR) in potato against *Phytophthora infestans*

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Keywords: systemic acquired resistance, L-arginine-conjugated chitosan nanoparticles, nitric oxide, *Solanum tuberosum*, *Phytophthora infestans*, pathogenesis-related genes

Systemic acquired resistance (SAR) is a plant's defense mechanism that protects against various pathogens after localized exposure to pathogens or chemical stimuli. The presented study was conducted to investigate the role of L-arginine-conjugated chitosan nanoparticles (ArgCSNP) as a potential inducer of SAR in potato (*Solanum tuberosum* L.) against *Phytophthora infestans* (Mont.) de Bary, the causal agent of late blight disease. For this purpose, the lower leaves of the potato cv. Bintje were treated with 0.5 mM ArgCSNP or 0.1% chitosan, and all the plants were then inoculated with *P. infestans*. Analyses were performed on both treated (local) and untreated (distal) leaves at 24 h after spraying with selected compounds (immunization) and at 6, 24, and 48 h after pathogen inoculation (hpi). The nitric oxide (NO) level was evaluated in the first stage as the key messenger of plant defense mechanisms. Electrochemical detection revealed significantly enhanced NO levels (approx. 4-fold) in response to ArgCSNP treatment; however, the increased signal formation was also observed in both local and distal leaves at 24 and 48 hpi. Subsequent experiments revealed that plant treatment with ArgCSNP resulted in an approx. 10-fold increase in *PR1* and *PR3* gene expression in distal leaves. Sequential treatment with ArgCSNP followed by *P. infestans* inoculation resulted in the upregulation of *PR2* and *PR3* at 48 hpi. Thus, the results suggest a potential role for ArgCSNP as a compound that can induce defense mechanisms favoring the acquisition of SAR.

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P-122

Redox-sensitive cysteine modifications regulate rice immunity during *Magnaporthe oryzae* infection

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Keywords: cysteine redoxome, post-translational modifications (PTMs), S-nitrosation, persulfidation, rice immunity

Post-translational modifications (PTMs) are critical regulators of protein function that not only affect the activity but also the synthesis, localization, and stability of target proteins. Among amino acid residues, cysteine is particularly reactive due to its thiol group and is a hotspot for redox-sensitive PTMs, collectively referred to as the cysteine redoxome. These modifications, especially nitric oxide (NO)-mediated S-nitrosation and hydrogen sulfide (H_2S) mediated persulfidation or S-sulfhydration, are increasingly recognized as important regulators of plant responses to oxidative stress. However, the precise roles of these modifications in modulating plant defense signaling remain poorly understood. In this study, we investigated the dynamics of NO and H_2S in rice (*Oryza sativa*) leaves during infection with *Magnaporthe oryzae*, the causative agent of rice blast disease. Two rice cultivars with contrasting responses to the pathogen, Dongjin (relatively resistant) and Nipponbare (susceptible), were used for comparative analysis. We quantified NO and H_2S levels, assessed the activity and expression of their biosynthetic enzymes, and evaluated their contributions to rice immune responses. To further elucidate the redox-based regulatory mechanisms, we employed a high-throughput proteomics approach to globally profile S-nitrosated and persulfidated proteins in infected rice leaves. Our analysis revealed numerous cysteine residues targeted by both modifications, highlighting a competitive interplay between NO- and

H_2S-mediated signaling pathways. These overlapping or mutually exclusive modifications appear to fine-tune protein activity in response to biotic stress. Taken together, our results provide novel insights into the redox-dependent post-translational regulation of rice immunity and highlight the importance of the cysteine redoxome in plant-pathogen interactions.

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P-123

Investigating ARWV2: HTS-based detection and RNAi involvement in rubbery wood disease

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Keywords: Apple rubbery wood virus 2, HTS, PAL

Apple (*Malus domestica*) is a globally significant fruit crop cultivated extensively across diverse agroecological regions. Apple trees are propagated vegetatively, facilitating frequent transmission and accumulation of viruses. Apple rubbery wood virus 2 (ARWV2) is a member of the genus Rubodvirus and Phenuiviridae family. Together with ARWV1, it was first identified in apple trees in Germany and the USA, showing symptoms of apple rubbery wood disease (ARWD), characterised by unusual flexibility of stems and branches. The flexibility (rubber-like feature) of the branches was shown to be caused by the decreased lignification of xylem vessels and fibres in the infected susceptible cultivars. ARWD was first reported in 1935 in England on apple later was found in other Rosaceae hosts, including quince and pear. It has a tripartite negative-sense single-stranded RNA (-ssRNA) genome comprising three segments: L encodes RNA-dependent RNA polymerase (RdRp), M movement protein (MP) and S capsid protein respectively.

High-throughput sequencing (HTS) technologies are universal diagnostic methods which can be used to detect and identify any known or unknown pathogens present in the investigated sample. In our previous work, we used HTS to survey the presence of pathogens in apple trees. Detailed bioinformatic analysis of those samples indicated the presence of ARWV2 in Freedom cultivar, which prompted a survey testing the possible presence of ARWV2 in apple, pear and quince trees.

A test of 77 trees using RT-PCR revealed the infection of 15 trees, including pear and quince, without showing any rubbery wood symptoms.

Using small RNA HTS, we profiled the small RNA pattern of ARWV2-infected and non-infected trees to investigate the differential expression pattern of vsiRNAs and miRNAs targeting the lignin biosynthetic pathway, particularly phenylalanine ammonia-lyase (PAL) genes. Based on this currently ongoing work, we are in the hope of better understanding the role of RNAi-based mechanisms in the ARWD symptom development.

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P-124

How does temperature affect the activity of RNA interference in woody and model plants?

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Keywords: temperature, RNA silencing, virus infection

RNA interference is a natural defence mechanism against viruses in plants. During viral infection, this defence system is activated and produces virus-derived small RNAs (21-24 nt long siRNAs) identical in sequence to the infecting virus. It has long been known that temperature strongly influences the plant-virus relationship and could affect both the miRNA and siRNA RNAi pathways, influencing the development of viral symptoms (Chellappan et al., 2005; Szittya et al., 2003). This relationship hasn't been well-characterised in herbaceous model plants, and has not been studied in woody hosts, such as fruit trees.

Our research investigated the effect of temperature on the infection of plum pox virus (Potyvirus plumpoxi - PPV) in two different hosts: a well-characterised model plant, *Nicotiana benthamiana,* and its natural host, *Prunus domestica*. *N. benthamiana* was infected by mechanical inoculation, while the plum trees were graft inoculated using sap or bud of a PPV-infected plum tree, respectively. The PPV infection was monitored in the *N. benthamiana* growing at ambient (22°C) and elevated (28°C) temperature conditions, while in the plum trees at greenhouse and field conditions. siRNA pattern was

characterised using high-throughput sequencing, while virus concentration was determined by real-time PCR. In the case of *N. benthamiana*, the concentration of PPV-derived siRNAs and the proportion of 24nt-long siRNAs increased, and the virus titre decreased at the elevated temperature in response to the 28 °C treatment. The size distribution profile of siRNAs of PPV-infected plum samples was similar under greenhouse and field conditions. The concentration of the PPV-derived siRNAs (22- 22nt) was higher in the field condition under extreme temperature conditions, which was positively correlated with the level of PPV titre.

More detailed analysis of the results is currently in progress and will contribute to a better understanding of the role of RNAi in PPV infection in response to temperature.

This work was supported by the grant of NKFIH PD_137621 and the Flagship Research Group Programme of the MATE.

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P-125

Suppressing non-host resistance of barley to tobacco mosaic virus and bacteria by heat shock and antioxidants

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Keywords: non-host resistance, heat shock, antioxidants

We have shown earlier that the symptomless non-host resistance of barley to wheat powdery mildew can be suppressed by combined heat shock & antioxidant treatments, resulting in reduced accumulation of the reactive oxygen species (ROS) superoxide ($O_2^{\bullet-}$), enhanced pathogen spread and development of hypersensitive-type local necrotic lesions (HR) (Künstler et al., 2018). Furthermore, the symptomless non-host resistance of barley to *Tobacco mosaic virus* (TMV) is also suppressed by heat shock (Király et al., 2021).

Here we elucidated 1/ the role of an early ROS (superoxide) burst in maintaining symptomless non-host resistance of barley to TMV at normal (20 °C) temperatures, 2/ how a combined heat shock & antioxidant treatment influences symptomless non-host resistance of barley to TMV. In TMV-inoculated barley (cv. Ingrid), heat shock pre-treatments (30 °C for 3 hours; 49 °C for 20 seconds, at 2 hours before TMV inoculation) resulted in significantly (50-100%) higher TMV titers than in untreated controls kept at 20 °C, confirming our earlier results. Plant resistance to virus infections may be conferred by an early ROS (e.g. superoxide) burst. We found that at 2 hours after inoculation superoxide production was essentially absent in heat shock pre-treated and TMV-infected barley leaves, as compared to controls kept at 20 °C (assayed by nitro blue tetrazolium /NBT/ staining). If superoxide indeed contributes to the non-host resistance of barley to TMV then its inhibition by antioxidants should suppress the resistance response (increase TMV levels). Infiltration of superoxide dismutase (SOD) and catalase (CAT) into barley leaves immediately after inoculation significantly (ca. 100 %) increased TMV levels as compared to control (buffer-infiltrated) plants, an effect similar to that of heat shock pre-treatments. Simultaneous application of heat shock (49 °C, 20 sec) and SOD+CAT infiltration caused an even higher increase in TMV titers and often the appearance of visible necrotic lesions resembling HR and indicating a programmed cell death response. Even when combined heat shock & antioxidant treatments did not result in visible HR, mesophyll cell death was detected in TMV-inoculated leaves associated with markedly elevated TMV levels.

We demonstrated that combined heat shock & antioxidant treatments result in a compromised non-host resistance of barley to TMV and the appearance of HR, pointing to the role of ROS (superoxide) in symptomless non-host resistance to viral infections.

Our future research will focus on effects of heat shock on resistance to bacterial infections.

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P-126

AtGSTU19 and *AtGSTU24* in *Arabidopsis thaliana* response to *Turnip mosaic virus*

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Keywords: glutathione, glutathione transferases, plant viruses, plant-innate immunity, resistance

Plants actively produce various types of molecules in response of change in the intercellular redox state. One of this molecules is glutathione which participates in the reactive oxygen species (ROS)-dependent signaling pathway, especially under biotic stress conditions. The glutathione itself is used by various enzymes to regulates plant cell redox state. In this context the glutathione S-transferases (GSTs) are glutathione-associated enzymes frequently induced in cells during the defense response of plants not only via highly specific glutathione-interaction abilities, but also by engagement in its signaling function. For our investigation we selected the *tau* class of GSTs which was previously reported to be upregulated as a response under stress conditions, but it's function in plant–pathogen interactions was unknown. Therefore, in this study, we validated the response of *Atgstu19* and *Atgstu24* knockout mutants to *Turnip mosaic virus* (TuMV) infection. Our results indicated systemic infection of TuMV was more dynamically promoted in *Atgstu19* mutants than in susceptible wild type (Col-0) plants, suggesting involvement *GSTU19* in TuMV resistance. On the other hand, in *Atgstu24* mutants level of the virus decreased. The virus infection in this mutants was also rarely associated with creation of TuMV particles. Moreover, further ultrastructural analyses of *Atgstu24* inoculated leaves revealed also the lack of virus cytoplasmic inclusions. These results revealed that *Atgstu24* mutants characterized resistance-like reaction to TuMV associated with influence on the virus infection cycle which created alternation in generation characteristic virus-associated structures. These results was also confirmed via analyses of expression of *GSTU24* which were upregulated in the *Atgstu19*–TuMV interaction. On the other hand, the upregulation of *GSTU19* highly correlated with virus limitation in the resistance-like reaction in the *Atgstu24*–TuMV interaction. Furthermore, the dynamic upregulation of GST and glutathione reductase (GR) activities resulted in significant induction (between 1 and 14 days post inoculation [dpi]) of the total glutathione pool (GSH + GSSG) in response to TuMV. On the contrary, in *Atgstu19*, which is susceptible to TuMV, GST and GR activity was induced only up to 7 dpi when symptom development was reported, which resulted in the induction of the total glutathione pool between 1 and 3 dpi. These observations indicated that *GSTU19* and *GSTU24* are important factors in modulating the response to TuMV and the role of glutathione in viral infection need to be subject of further analyses.

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P-127

Red light irradiation at night enhances tomato defence against fungal pathogen *Botrytis cinerea*

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Keywords: plant immunity, RNAseq, RT-qPCR, ROS

Fungal pathogens pose a serious threat to global agricultural production accounting for approximately a 10-20% reduction in crop yield, and contributing to losses post-harvest, thereby not only representing a major constraint to food production, but also negatively influencing food quality. Among these pathogens *Botrytis cinerea* - commonly known as grey mould – is particularly problematic, due to its wide range of hosts and high adaptability to adverse conditions. Tomato (*Solanum lycopersicum* L.) is among the most economically significant vegetable crops, valued for both its nutritional composition and culinary versatility. It is also one of the primary hosts targeted by this destructive fungal pathogen. Numerous studies have shown that light not only serves as an energy source but also has immunity-enhancing capabilities. Red light, in particular,

is known to enhance plant defence, however the underlying molecular machinery behind this effect is not fully understood. Our study aimed to uncover the plant defence mechanism regulated by red light, which was applied at night, when most phytopathogens are highly infectious. We examined the accumulation of key reactive oxygen species (ROS) involved in plant immune responses, alongside the effects of red light on the activity of major antioxidant and detoxification enzymes, including catalase, ascorbate peroxidase, peroxidase, and glutathione S-transferase. To further elucidate the impact of red light on plant defence mechanisms, we quantified key defence-related phytohormones–salicylic acid, jasmonic acid, and abscisic acid–following nocturnal red-light exposure. To gain a clearer understanding of the changes that occur on gene expression levels, we utilized RNAseq together with RT-qPCR to further validate our results. We successfully identified several upregulated circadian clock related genes, alongside with numerous plant defence genes that showed elevated expression after half an hour of red light irradiation starting from midnight. In addition the expression levels of several transcription factors were also elevated after the treatment. Notably, extended red-light exposure over one week made the plants more successful in fighting off *Botrytis cinerea* infection, and had a positive effect on key antioxidant enzymes both on gene expression and on enzymatic activity levels. These findings together suggest, that nocturnal red light treatment can prime defence responses through circadian and redox-regulated pathways.

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P-128

Selection and silencing of *WRKY* and *MAP kinase* family members to test their potential in resistance breeding to *Ralstonia solanacearum* infection in potato

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Keywords: transcription factors, phytopathogens, negative regulators

Ralstonia solanacearum (*Rs*), is one of the most important soilborne phytopathogens causing bacterial wilt disease in many plant species, including Solanaceae crops, such as potato, tomato, etc., resulting in severe crop loss throughout the globe. The root is the main entry point for *Rs*. WRKY transcription factors are critical players in modulating plant resistance to phytopathogens. However, little is known about MAP kinase (MAPK) signalling and WRKY transcription factors-mediated defense in potato in response to *Rs* infection. Previously, it was reported that *WRKY22* and *WRKY24* were downregulated in the *Rs*-resistant potato cultivars 'Calalo Gaspar' (CG) and 'Cruza 148' (CR) upon *Rs* infection. It was also found that MAPK kinases *MAPK9* and *MEKK1* in CG, *MEKK7* in CR, and *MEKK EDR1* in both cultivars of potato are downregulated by *Rs* infection. Thus, their role might be negative concerning pathogen defense in potato (Jose et al. 2023). Our work aims to test the role of MAP kinase signalling and *WRKY22* and *WRKY24* during *Rs* infection. To achieve this goal, we are using the technique of gene silencing. Fragments of the selected genes were PCR amplified and cloned in the binary vector pCP60 behind the constitutive *35S* promoter in antisense orientation to provide strong expression for the antisense mRNA. The *WRKY22, WRKY24,* and *MAPK9* constructs are already transformed into the *Rs*-susceptible potato cultivar, 'Désirée', mediated by *Agrobacterium*. We are studying the level of gene silencing in transgenic plants by RT-qPCR to select for the plants with the highest repression. The selected plants will be propagated *in vitro* and tested for *Rs* resistance. Cloning and transformation of *MEKK1*, *MEKK7,* and *MEKK EDR1* fragments are in progress. The information generated by this study may facilitate molecular breeding of *Rs*-resistant potato varieties not only by antisense silencing, but also by gene editing.

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P-129

Plant immune system is stimulated by root microbiome against root-knot nematodes (RKNs) in vegetable plants

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Keywords: Plant immune system, RKNs, root microbiome

Background: Plant Microbiome is the collective communities of plant-associated microorganisms, also referred as plant second genome. Plants select beneficial rhizosphere microbes in naturally microbiome rich soils. Microbiome-generating formulations, containing arbuscular mycorrhizal fungi (AMF), plant growth promoting rhizobacteria (PGPR) and other beneficial microorganisms, can be added to experimental soils to test their effectiveness in stimulating plant defense against soil-borne parasites, such as RKNs: to understand the basis of the relationship between specific microbiomes and plant defense is the goal of our research activity.

Key Findings: Commercial microbiome-generating formulations and/or green composts have been used to treat tomato plants grown in pots or large plastic trays under controlled conditions in a glasshouse. Pre-treatments with specific doses of these formulations lowered both infection level and reproduction rate of RKNs which were artificially inoculated a few days after treatments. The inhibitory effect on nematode infection was always associated with a large mycorrhization of roots in the rhizosphere. Plants were primed by mycorrhization and at the onset of nematode attack the expression of genes markers of plant immune response, such as PR4b, was found to markedly increase. PR4b over-expression is indicative of the hypersensitive cell death induction and it is generally a marker of the execution of plant immunity following a biotic challenge. Water extracts of the used formulations were proved not to be toxic to nematode juveniles [1].

Future Research Directions: Future studies will focus on the attempts to enhance the performance of microbiome-generating formulations and/or low scale composts by identification and selection of most performing AMF strains.

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P-130

The pathogen-inducible PRLIP genes negatively regulate defence responses in *Arabidopsis thaliana*

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A novel plant-specific pathogenesis-related gene family encoding lipase-like proteins (PRLIPs) was identified earlier in our group by a differential screening of Arabidopsis plants pretreated with the resistance-inducer β-aminobutyric acid (BABA). BABA is a non-protein amino acid that induces resistance to pathogens by priming different defence mechanisms in plants. In Arabidopsis the gene family consists of six tandem repeated members on the 5th chromosome (PRLIP1, PRLIP2, PRLIP4, PRLIP5, PRLIP6, and PRLIP7) and three distantly related, constantly expressed genes (PRLIP3, PRLIP9, and PRLIP8). The catalytic triad of serine, aspartic acid, and histidine amino acids characteristic of GDSL lipase (class III) proteins is conserved among all PRLIPs, suggesting that these proteins have esterase or lipase activity. During pathogenesis, PRLIP1 and PRLIP2 are highly induced. Therefore, we examined the effect of these two inducible PRLIP genes on the defence responses and the BABA-induced priming of Arabidopsis during infection by a virulent strain of a hemibiothrophic bacterium, *Pseudomonas syringae* pv tomato (Pst).

We found that in plants PRLIP2 deficiency and PRLIP1 overexpression cause very similar phenotypes. On one hand, they are impaired in SA-dependent defence responses. On the other hand, the increased growth and delayed senescence under non-stress condition indicated an augmented auxin content of these plants. Since the antagonism of auxin and SA signalling is well documented, increased auxin levels in these plants may explain the suppression of SA-dependent responses. Although the significant role of PRLIP1 and PRLIP2 as regulators of the free auxin pool in Arabidopsis was clearly demonstrated the exact molecular background of this phenomenon leading to divergent hormonal balance is still under investigation. In the plant, the level of the biologically active form of auxin, free indole acetic acid (IAA), is regulated by two opposite processes, its

synthesis, and its conjugation to amino acids and/or sugars. We hypothesize that PRLIP1 may be the as-yet-unknown esterase that hydrolyses the sugar from indole-acetic acid glucoside and renders it biologically active. Our results indicate that PRLIP1 plays an important role in regulating the free IAA pool in Arabidopsis and thereby fine-tuning plant growth and defence responses.

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P-131

Pipecolic acid-triggered immunity in tomato: The role of ethylene signaling

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Keywords: pipecolic acid, ethylene, plant immunity

Infection by phytopathogens triggers complex local and systemic defence mechanisms in plants, involving the production of mobile signaling compounds crucial for survival. Pipecolic acid (Pip) is a key mediator of systemic resistance, which is typically associated with defence hormones like salicylic acid (SA); nonetheless, its potential interplay with ethylene (ET) remains unexplored. In this study, we investigated the role of ET signaling in Pip-induced local and systemic defense responses in tomato leaves, following 6- and 24-hour Pip pretreatments. Our results show that Pip had no significant effect on reactive oxygen species (ROS) metabolism after 6 hours; however, after 24 hours, it promoted the systemic accumulation of superoxide, independently of active ET signaling. After 6 hours of Pip treatment, a local increase in guaiacol peroxidase (POD) activity was observed in both genotypes. However, by 24 hours, a significant elevation in POD activity was detected exclusively in the leaves of wild-type (WT) plants, while superoxide dismutase (SOD) activity was enhanced in both lines. In addition, systemic expression of the SA marker gene *PR1* was significantly upregulated in WT leaves, whereas no such induction was detected in the *Nr* mutant. Although Pip strongly elevated *ERF1* transcript levels in both genotypes, a notable rise in *DEF9* expression was observed exclusively in *Nr* plants. Moreover, Pip triggered ET production in the leaves of WT plants, contributing to enhanced systemic resistance to *Botrytis cinerea* as evidenced by a reduction in lesion size, furthermore, it also promoted resistance against *Pseudomonas syringae* infection. Based on these results, ET appears to play a regulatory role in the modulation of Pip-induced systemic defense responses, which can positively influence the establishment of resistance upon subsequent pathogen infection.

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P-132

How phytochromes influence the *Pseudomonas syringae*-induced ER stress response in tomato leaves?

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Keywords: light, stress, ER stress, ROS, phytochromes

Light plays a key role not only in the normal plant growth and development, but also in defence responses. During pathogen attacks, excessive protein synthesis can overwhelm the protein-folding capacity of the endoplasmic reticulum (ER), leading to ER stress. Although crosstalk between light signalling and ER stress responses has been reported, the molecular mechanisms underlying this crosstalk are poorly understood, especially in the systemic responses of plants. Infection with *Pseudomonas syringae* induced a rapid production of hydrogen peroxide but not superoxide in leaves, which was monitored in time-course experiments during different day/night periods. Within 24 hours, the expression of the salicylic acid marker gene *PR1* was significantly induced both locally and systemically but it was lower in the systemic leaves. The expression of the ER stress and unfolded protein response (UPR) marker gene *luminal binding protein* (*BiP*) was also rapidly induced. Using photoreceptor *phytochrome A (phyA), B1B2 (phyB1B2)* and *AB1B2* (*phyAB1B2*) mutants, the role of phytochromes in ER stress and the UPR was demonstrated, as the expression of *PR1* and *BiP* was lower in the mutants and dependent on the presence of light, while pathogen infection was more significant as compared to wild-type leaves. These

results suggest that phytochromes integrate light signalling with the UPR to alleviate ER stress and regulate plant defence responses.

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P-134

Comparative analysis of the 2b suppressor proteins of cucumber mosaic virus (CMV) variants infecting various weed species

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Keywords: viral suppressor, agroinfiltration, CMV

The cucumber mosaic virus (CMV) can infect over a thousand plant species, including numerous important horticultural crops such as pepper, tomato, cucumber, and zucchini. With the help of polyphagous vectors, CMV can be easily spread from weeds, which could serve as intermediate hosts and secondary food sources for vectors, to cultivated plants. During a viral infection, the plant's defence system, RNA interference (RNAi), is activated. To evade this mechanism, viruses have developed proteins that function as viral suppressors of RNAi (VSRs). CMV possesses such a VSR: the well-characterised 2b protein.

The study aimed to isolate, sequence and analyse the 2b proteins from CMV variants infecting common milkweed (*Asclepias syriaca*) and black nightshade (*Solanum nigrum*) collected from five different locations. Additionally, we aimed to examine the VSR activity of these different 2b protein variants and to investigate how they differ in sequence and suppressor activity compared to the well-characterised 2b proteins encoded by CMV strains infecting cultivated crops.

The NCBI GenBank database contains 341 variants of the 2b coding ORF, whose sequences were compared and analysed for their sequence variation, geographical distribution and host origin. Based on multiple sequence alignment, primers were designed to successfully amplify 2b coding regions from different phylogenetic groups. The PCR-amplified products were cloned into pJET vector and sent for Sanger sequencing. Based on our results, the 2b proteins of weed-infecting CMV variants cluster into the IA and II subgroups of the virus. Bioinformatic analysis revealed that, although the amino acids associated with VSR function are conserved in these variants, several substitutions are present which may potentially influence their suppressor activity. To check any possible difference in the VSR activity of these 2b proteins, *Agrobacterium tumefaciens*–mediated transient gene expression assay has been initiated. In our ongoing research, we are investigating both local and systemic VSR activity of the 2b proteins from CMV variants infecting weed species.

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P-135

Identification the regulatory elements in *SP6A* promoter to improve tuberization in the potato cultivar 'Désirée'

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Keywords: potato tuberization, SP6A gene, transcriptional regulation

Tuber formation in potato (*Solanum tuberosum* L.) is a complex developmental process regulated by the *SP6A* gene, a mobile tuberization signal, and a homolog of the *Arabidopsis* florigen *FT*. *SP6A* expression is negatively affected by elevated temperature and stresses and also by long-day conditions in the short-day tuberizing potatoes. In day-length independent tuberizing cultivated varieties, such as 'Désirée', *SP6A* expression is reduced under stress, resulting in decreased tuber yield. Overexpression of *SP6A* increases earliness of tuberization but reduces shoot growth and increases tuber number. We hypothesize that fine-tuning on *SP6A* expression would increase the earliness of tuberisation and stress tolerance of cultivated potatoes without a significant effect on canopy development and tuber number. To test this hypothesis we identified the most homologous *SP6A* allele in Désirée by designing PCR primers based on the *S. phureja* genome sequence (https://spuddb.uga.edu/) with the highest homology to the Neo-Tuberosum *SP6A*. Five distinct fragments of the *SP6A* promoter region were isolated, amplified, cloned, sequenced, and compared to the *SP6A* promoter region of *S. phureja*, followed by in silico analysis to detect potential transcription factor binding sites. Sequence alignment of the 2-kb *SP6A* promoter region revealed a high degree of conservation between 'Désirée' and *S. phureja*, with several single nucleotide polymorphisms and small insertions/deletions. Notably, some polymorphisms were located near the predicted transcription factor binding sites, which could have functional consequences on promoter activity under different environmental conditions. Multiple regulatory motifs in the 'Désirée' *SP6A* promoter were identified using the Plant Transcriptional Regulatory Map database (https://plantregmap.gao-lab.org/) including Dof, ARF, E2F/DP, and MYB-related elements. However, a comparative analysis revealed key differences: Désirée lacked SBP binding sites detected in *S. phureja*, while having additional M-type MADS-box and Nin-like transcription factor binding sites. To test the activity of the in silico identified promoter regions in planta the PCR products were cloned into the pCAMBIA1391Z GUS fusion vector and will be transformed into 'Désirée'. Plant genes are generally under positive/negative regulation. If this is the case also for *SP6A*, the binding site(s) for negative regulators will be eliminated with targeted mutagenesis. We expect that these modifications will lead to earlier tuber initiation under suboptimal conditions offering a promising strategy for developing climate-resilient potato cultivars.

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P-136

Molecular insights into the genes responsible for stenospermocarpy in pitaya (*Hylocereus undatus, Cactaceae*)

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Keywords: aborted seeds, ovule development, regulatory pathways

Stenospermocarpy, the development of seedless fruit due to aborted seeds, is a valuable trait in many fruit crops. There is limited information about the molecular and genetic aspects of stenospermocarpy in Cactaceae, especially in pitaya (*Hylocereus* species), including the pathway to seed development. A mutant, line #8-26 has been identified that produces fruit with mainly aborted seeds. This spontaneous mutant is an offspring of the diploid *H. undatus* (line #89-024). Our study aims to compare the transcriptomes of these two lines, i.e., the maternal line #89-024 and the mutant #8-26, to elucidate the genetic mechanisms behind normal seed

development and seed abortion in pitaya. Through RNA sequencing and bioinformatic analysis, this research identified several putative key genes, such as ACS7, EIL1, RING U-Box superfamily protein, HVA22-like protein, BURP domain-containing protein, that are involved in ABA and ethylene cross talk. Our results show that ethylene signaling represses some of the ABA responsive genes resulting in the arrest of seed development and the production of non-viable seeds. These findings will contribute to the understanding of stenospermocarpy in pitaya, a cactus fruit crop, as well as other perennial species, having significant implications for breeding programs aimed at improving fruit quality in pitaya and other crops.

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P-137

Role of *in silico* methods through revealing the effects of molecular interactions between biomolecules and ligands in prediction of protein functions

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Recent advancements in informatics have made widespread application of *in silico* methods for biological research possible. Introduction of AI-based algorithms and improvement of computer performance specifically let us make more accurate predictions of how supra- and infra-individual biological systems work.

The functionality and structural stability of proteins heavily depends on the possible intra- or intermolecular interactions between the amino acid sidechains and other ligands or heteroatoms. Changing one or more amino acid with mutation can change the whole protein's functionality, blocking certain interactions or making them possible.

Computational methods let us not just model the 3 dimensional structure of proteins, but also understand the interactions that stabilize these structures. Therefore, the functional difference between protein variants can be predicted with them.

Several of these methods are usually combined to build system that can be used to simulate molecular interactions. Molecule modelling lets us predict the secondary and tertiary structure of proteins, and with docking, we can assemble several peptide chains into a protein complex. Molecular dynamics can be used to simulate the movement and gyration of the molecule's atoms and bonds in an explicit solvent.

Below are three examples of *in silico* modelling of the molecular interactions of proteins:

1) Dimerization of *Brachypodium distachyon* LBD transcription factors. In a previous work, we started to model the LBD protein family of *Brachypodium distachyon* (purple false brome), a model organism of monocot plants. Molecular dynamics was used to determine whether the examined proteins function as homo- or heterodimers (and in the latter case, which other LBD protein they dimerize with).

2) The simulation of certain mutations in the Rubisco activase enzyme of *Zea mays*. Ribulose-1,5-bisphosphate (RuBP) carboxylase-oxygenase (Rubisco) enzyme is the limiting step of photosynthetic carbon fixation, and its activation is regulated by its co-evolved chaperone, Rubisco activase (Rca). We were investigating the distal effects of mutations in the conserved Walker A and Walker B regions of the ATPase on the hexameric structure of the protein, that were also important for the increased Rubisco activase function of the mutant proteins at elevated temperature.

3) The investigation of certain phosporylation sites of the E2F-DP-RBR protein complex of *Arabidopsis thaliana*. It was previously demonstrated that hyper-phosphorylation of RB controls its interaction with E2F and inhibits its tumor suppressor properties. In silico methods can be used to reveal how RB activation signals are integrated in a phosphorylation code that determines the diversity of RB activity.

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P-138

Proteomic perspective of trap leaf development and prey digestion in carnivorous plant - forked sundew

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Keywords: adhesive trap, Drosera binata, hydrolases, proteomics

Carnivorous plants are remarkably adapted to thrive in acidic soils with low nutrient availability. This adaptation is significantly enhanced by specialized trap leaves that attract, capture, and digest prey. Our target species, forked sundew (*Drosera binata*), features distinctive forked leaves covered with secretory glandular tentacles containing a cocktail of various hydrolytic enzymes. We conducted a proteome profiling across 5 leaf developmental stages to elucidate key proteins contributing to the ontogenesis of sundew traps. Next, we performed induction experiments using wingless flies and mock prey, specifically a mixture of chitin (N-acetylglucosamine polymer) and pachyman (β-1,3-glucan), to profile the digestive enzymes. Total proteins were extracted using a detergent-containing buffer, purified, and digested by trypsin using a single-pot solid-phase-enhanced sample preparation method, followed by quantification by nano liquid chromatography coupled with mass spectrometry. Overall, we identified 1,183 proteins in developing traps and detected 485 differentially accumulated proteins with ANOVA at $Q < 0.001$. In a parallel study involving flies and mock prey trap induction, we identified 2,021 proteins and revealed 342 differentially accumulated ones (ANOVA at $Q < 0.01$). Principal component analysis of quantified proteins demonstrated well-separated groups corresponding to each sequential developmental stage and individual induction treatment. Furthermore, we identified 91 hydrolases in developing leaves and 101 in the induced traps, with vignain endopeptidase being the most reactive in both induced trap treatments. Further exploration of these identified proteins, particularly the hydrolytic enzymes, will shed light on their functional roles in digestion and potential biotechnological applications.

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Fruit thinning improves fruit size and return bloom in cranberry (*Vaccinium macrocarpon*) in high tunnel soilless cultivation

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Keywords: anthocyanin, crop load adjustment, fruit size

American cranberries produce many flowers, but few of them develop into mature fruit by the end of the growing season. Limited information is available on cranberries crop load management; therefore, this study evaluated the effects of fruit thinning during early fruit development on fruit quality and return bloom in two cranberry cultivars, 'Pilgrim' and 'Stevens.' The four treatments included (1) removing all fruits on the upright (0F), (2) leaving the lowermost fruit (1F), (3) leaving two lowermost fruits (2F), and (4) no fruit removal (control). The experimental plants were arranged in a randomized complete block design consisting of six blocks, each with eight replicate uprights.

Results showed that fruit thinning treatments and cultivar significantly affected fruit size with 'Stevens' producing larger fruit than 'Pilgrim'. In addition, the weight of a single fruit in 1F was equal to 2F in 'Stevens', while in 'Pilgrim' 1F generated heavier single fruit as compared to other treatments. The control treatment resulted in more developed berries per upright in 'Stevens' than in 'Pilgrim'. Furthermore, thinning treatments increased fruit anthocyanin concentration in 'Pilgrim', and increased return bloom in the following season through reduced reproductive burden. These findings suggest that fruit thinning is an effective approach for increasing the cranberry fruit size. Furthermore, it improves return bloom in the flowering uprights in the subsequent year.

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P-140

Identification of peroxisomal proteins as putative interactors of the floral regulators FLOWERING LOCUS T and TERMINAL FLOWER 1

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Keywords: floral transition, protein interactors, peroxisomal proteins

FLOWERING LOCUS T (FT) and TERMINAL FLOWER 1 (TFL1) are two key regulators of floral transition and inflorescence architecture. Although they both belong to the PHOSPHATIDYLETHANOLAMINE BINDING PROTEINS (PEBP) family and share about 60% identity in their polypeptide sequence, they have antagonistic functions: FT promotes flowering while TFL1 represses floral transition and prevents the determination of the inflorescence. In order to identify regulatory mechanisms that contribute to their functional antagonism, we used affinity purification coupled with mass spectrometry (AP-MS) to identify new protein interactors of FT and TFL1. The experiments were carried out with *Arabidopsis thaliana* seedlings over-expressing tagged versions of FT or TFL1. Surprisingly, we co-purified several peroxisomal proteins as potential interactors of TFL1. A reverse genetics analysis was then conducted to test the functional relevance of these proteins in the regulation of flowering. Among all mutants tested, we found that *cat2-3*, a mutant in the *CATALASE2* (*CAT2*) gene, was late flowering in short days, but this phenotype was much weaker in other *cat2* alleles (*cat2-1* and *cat2-2*). Bimolecular fluorescence complementation (BiFC) and yeast two-hybrid (Y2H) assays were performed and indicate that CAT2 could physically interact with TFL1 and FT. These results suggest that CAT2 may interfere with the regulation of flowering.

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P-141

Revisiting the impact of the *CROWN ROOTLESS 5* mutation on global rice plant development and architecture

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Keywords: rice, development, branching, AP2 transcription factor

Branching is an essential determinant of rice plant architecture that determines agronomic performance of plants. In rice, branching events giving rise to crown and lateral roots, tillers, and panicle branches play important roles in determining overall plant productivity and adaptability to abiotic stresses. The *CROWN ROOTLESS 5* (*CRL5*) gene, first reported by Kitomi et al. (2011), is essential for crown root formation and development. This gene belongs to the *APETALA2* transcription factors family and has been also described as *PLETHORA8* (*PLT8*) by Luong et al 2021. Compared to wild-type (WT) plants, the *crl5* mutant exhibits a marked reduction in crown roots due to the failure of crown root primordia initiation and the *plt8* mutant is characterized by a significant decrease in the number of primary panicle branches This suggests that the CRL5/PLT8 transcription factor is involved in different branching events.

To investigate if this transcription factor could be involved in other branching events, we refined the phenotyping of *crl5* mutant plants at different developmental stages. Analysis of one-month-old plants confirmed the reduced crown root phenotype and revealed a significant reduction in tiller number in *crl5* mutants compared to WT. The mutation didn't affected the lateral root density in crown roots whereas lateral root length was increased in *crl5* plants. Crown roots and tiller number decrease persisted at four months, along with a noticeable reduction in plant height. We also confirmed that panicle has fewer primary branches and an increased number of secondary branches per primary branch in the *crl5* mutant.

Taking all together this confirmed the involvement of CRL5 in crown root formation as well as in panicle branching. In addition, our data suggests that CRL5 is also involved in tiller formation, but not in lateral root

formation even if it could play a role in lateral root growth. This suggests that this transcription factor may have been recruited early during evolution to control different *shoot-associated branching* events but not root associated branching leading to lateral roots.

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P-142

PIB/CIB3 is a potential mediator of lignification in *Arabidopsis*

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Keywords: lignification, lignin-biosynthetic gene, transcription factor, bHLH, Arabidopsis

Lignification is a resource intensive process involving deposition of lignin, a complex phenolic polymer, into the secondary cell walls of plant tissues. It plays a pivotal role in providing structural support, impermeability, and defence against various environmental stresses and pathogens. Understanding the regulatory mechanisms governing lignin biosynthesis is of interest for improving plant biomass utilization and enhancing stress tolerance in crops.

We previously reported PIRIN2 (PRN2) as a suppressor of xylem vessel lignification in Arabidopsis vascular tissues. To further investigate the regulatory network controlling lignification, we identified a PRN2-interacting basic helix-loop-helix protein (bHLH) using yeast-two-hybrid, which was also confirmed with co-immunoprecipitation assays. We named it PIB, but it is also known as CRYPTOCHROME2-interacting bHLH3 (CIB3).

We observed that *PIB*, together with its close homolog *PIB-LIKE/CIB2*, plays a crucial role in plant development, as double knockout mutants are gametophytic lethal. Detection of *PIB* promoter activities indicated that *PIB* is expressed in various organs and tissue types throughout the plant's life cycle, while the PIB protein localizes to the nucleus. Transcriptomic analyses revealed a potential role for PIB in regulating the diurnal expression patterns of lignin-biosynthetic genes. Additionally, our transient expression assays supported that PIB can suppress the promoter activity of some of these genes, prompting further investigation into the underlying mechanisms. Although we could observe changes in the diurnal expression patterns of some of the lignin-biosynthetic genes in the *pib* mutated genotypes, the alterations in the lignin content or composition were opposite and less prominent than in the *prn2-2* mutant, as suggested by pyrolysis-gas chromatography/mass spectrometry and acetyl bromide soluble lignin assays. Altogether, our findings propose that PIB may mediate the diurnal regulation of lignin biosynthesis, and we are further investigating its potential role in lignification.

P-143

Maculae are multifunctional structures in *Ficus citrifolia* Mill. (Moraceae) supporting fig tree-fig wasp mutualism

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Keywords: plant microscopy, plant-insect interaction, physiology

Fig trees represent a group of great ecological importance within the Moraceae family, notable for their unique reproductive structure, the fig, which can refer to both an inflorescence and an infructescence. As an inflorescence, the fig consists of a cluster of internally enclosed, inconspicuous flowers, with a perianth typically reduced to a few sepals. Fig trees are pollinated through a highly specialized mutualistic interaction with fig

wasps, which use the fig as a site for oviposition (Pereira 2024). In many fig species, the external surface of the fig displays maculae, regions marked by brownish or yellowish pigmentation, particularly evident during the pistillate flower receptivity phase (Verkerke 1986). This study aimed to investigate the structure and function of maculae in *Ficus citrifolia* Mill., a Brazilian forest species, through detailed morpho-physiological analyses across all stages of the fig reproductive cycle. Figs were collected at different reproductive phases and processed for surface, histological, and ultrastructural analysis; measurements of internal fig temperature and counts of oviposition scars from parasitic wasps were also conducted. Maculae exhibited an epidermis with active stomata, tector and secretory trichomes, and a subepidermal layer of parenchymatic cells containing druses or phenolic compounds. Significant increase in the number of stomata, fibers, and sugary secretions were observed during the pollen release phase, with secretions confirmed by Fehling's and PAS staining. Figs maintained a lower internal temperature than surrounding plant tissues during the hottest part of the day, particularly during pollen release phase. Parasitic wasps preferentially inserted ovipositors into fig areas without maculae, especially in larger figs. The abundance of stomata contributes to internal temperature regulation, ensuring a cooler microenvironment favorable for the development of pollinating wasp larvae. The sugary secretion attracts patrolling ants that may offer biological protection against herbivores and potential fig predators. Furthermore, the accumulation of phenolic compounds in the maculae appears to serve as a chemical defense mechanism, deterring parasitic wasps, as evidenced by their avoidance of maculae regions during oviposition. Altogether, these combined structural and functional traits reinforce the importance of maculae in supporting the fig-wasp mutualistic interaction.

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PLANT NANOBIOLOGY

P-144

Role of organic nanofibers on enzyme antioxidant defence system in *Stevia rebaudiana* plantlets

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Keywords: *Stevia rebaudiana* Bert., enzyme antioxidants, *in vitro* propagation, IAA

Plants are exposed to stress conditions (low gas exchange, high humidity, low light, etc.) during *in vitro* propagation by direct organogenesis. Many research studies have shown that vitamins and amino acids may support plants to reduce the adverse effects of biotic and abiotic stressors. The branched-chain amino acids as valine, are essential for plant protein synthesis. The exogenous application of nicotinic acid (NA) could significantly enhance plant growth and drought stress tolerance. In this work, we chose organic peptidomimetics as an auxin indole-3-acetic acid (IAA) delivery system to study the effect of nanofibers on *in vitro Stevia rebaudiana* Bert. propagation and enzyme antioxidant activity. For the purpose of the study, as a carrier of IAA, we used an organic compound, that includes two fragments of valine and nicotinic acid linked together and doubled through diamino hexane spacer and possessed the capability to produce nanofibrilar networks in organic solution and also in the absence of solvent. The analysis of the obtained results showed that the resulting organic nanofibers alone (M6) or as an IAA carrier (M6+IAA) added to the MS medium during direct organogenesis, positively affected growth parameters (fresh and dry biomass accumulation, shoot height, shoot number per explant, number of nodes per shoot and micropropagation rate) of S. rebaudiana. The antioxidant enzyme activity bioassay test demonstrated enhanced SOD activity only, while the CAT, APX and GPX activity was reduced. Also, the root formation percent was higher in plants treated with nanofibers. The results showed that M6 enhanced plantlet growth parameters at a higher level than M6-IAA, but the rooting was higher at M6-IAA treatment. The amino acid valine, the nicotinic

acid and the auxin IAA combined influence the growth and antioxidant activity.

These results showed that those organic nanofibers could serve as an IAA carrier with lower environmental impact, comparable effect, and effective plantlet growth.

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P-145

Effects of seed priming with different-sized ZnO nanoparticles on growth and reactive molecule levels on zinc deficient tomato (*Solanum lycopersicum* L. CV. Mano)

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Keywords: Zn deficiency, *Solanum lycopersicum*, nanoparticle, seed priming

Zinc (Zn) is the second most abundant transition metal and is essential for living organisms. Zn-deficient soils are widespread globally, including in Hungary. This study investigated the effects of ZnO nanoparticles of two different sizes (~5 nm and ~40 nm) as seed priming agents at a concentration of 100 mg/L on *Solanum lycopersicum* L. cv. Mano growing in Zn-deficient nutrient solution. Tomato seeds were primed for 24 hours and germinated in a nutrient solution with $ZnSO_4$ (Zn+) or without $ZnSO_4$ (Zn-) for 3 weeks. Sensitivity to Zn supply was evaluated by analyzing Zn levels and localization, organ growth parameters (fresh and dry weights of shoots and roots, shoot and root lengths, leaf area), and reactive oxygen and nitrogen species. Fluorescent probes were used to detect nitric oxide (NO), hydrogen peroxide (H_2O_2), and peroxynitrite ($ONOO^-$) levels in the root system. Three-weeks-long treatments successfully induced Zn deficiency in *Solanum lycopersicum* indicated by declined biomass production. Regarding reactive molecules, Zn supply decreased NO and H_2O_2 levels and increased $ONOO^-$ levels in roots. Leaf area, shoot and root biomass were improved by 5 nm and 40 nm nZnO seed treatments compared to the hydroprimed plants. Overall, seed nanopriming using ZnO NPs is a promising approach for biomass improvement of tomato in Zn-deficient environment.

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P-146

Nitric oxide delivering nanoparticle as pretreatment strategy against *Botrytis cinerea* in tomato fruit

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Keywords: nitric oxide, S-nitrosoglutathione, chitosan, nanodelivery, *Solanum lycopersicum*, *Botrytis cinerea*

Fungal pathogens are a major global threat to agriculture, causing around 70–80% of all plant diseases and leading to significant economic losses. *Botrytis cinerea* is one of the most harmful fungal pathogens, a necrotrophic and polyphagous species that infects over 200 plant species, including tomatoes, causing significant losses in both yield and quality. Infected tomatoes often become unmarketable. One promising, sustainable solution lies in nanotechnology, particularly in systems that deliver nitric oxide (NO), a molecule known to play a key role in plant defense. Its natural derivative, *S*-nitrosoglutathione (GSNO), acts as an effective NO donor. By encapsulating GSNO in biodegradable chitosan (CHT) nanoparticles, we can achieve targeted and controlled NO release. Chitosan itself also has antimicrobial properties, making it a double-acting defense tool. We tested whether this nano-based approach works in protecting tomato fruits (*Solanum lycopersicum* L. cv. Moneymaker) against fungal infection. Before exposing the fruits to *B. cinerea* (strain B05.10, at 10^6 conidia/ml), we pre-treated them with distilled water (as a control), 5 mM free GSNO, chitosan-encapsulated GSNO nanoparticles (NPs), or empty chitosan NPs. Three days post-inoculation, infection symptoms were evaluated by measuring lesion diameter (mm), calculating disease incidence (%), and visualizing fungal hyphae. Furthermore, the lycopene content of the tomato fruits was also quantified. The results were clear: tomatoes treated with the GSNO-loaded NPs showed

significantly less infection compared to those treated with free GSNO or empty chitosan particles. This suggests that nanoencapsulation not only improves NO delivery, but also enhances the antimicrobial effect offering a promising tool for sustainable plant protection.

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P-147

Effect of B_1-vitamin stabilized copper nanoclusters on the viability and ROS content of maize protoplasts

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Keywords: maize, B_1-vitamin stabilized copper nanocluster, protoplast, viability, ROS content, cationic polymer

Maize protoplasts can serve as valuable material for precise genome editing methods, such as targeted nucleotide substitution with synthetic oligonucleotides alone (Oligonucleotide Directed Mutagenesis) or in combination with CRISPR system. The success and efficiency of genome editing can be significantly influenced by the viability of maize protoplasts and the presence of reactive oxygen species (ROS). Previous studies have demonstrated that silica (SiO_2) and gold nanoparticles (AuNPs) can have positive effects on protoplast cultures. Furthermore, B_1 vitamin-gold nanoparticles have been shown to possess stronger antioxidant capacity than pure vitamin B_1.

Copper is an essential micronutrient; therefore, its vitamin B1-stabilized nanoparticles (B1CuNPs) were chemically synthesized, characterized, and their effect compared to vitamin B1 was investigated on the viability and ROS content of SZ22 (capable of regeneration) maize protoplasts in the presence of cationic polymer used in our laboratory for transformation. Vitamin B_1 and B1CuNP were added at concentrations ranging from 20 to 320 µM to protoplasts in R medium containing 0.75 M mannitol. 20 µg/ml of a cationic polymer was added to each sample. ROS content was determined by measuring the fluorescence of 5(6)-Carboxy-2′,7′-dichlorofluorescein diacetate (H2DCFDA) dye. Fluorescein diacetate/propidium iodide (FDA/PI) assay was performed to evaluate protoplast viability. ROS content of maize protoplasts under the conditions of transformation decreased upon the addition of either vitamin B1 or its complex with copper nanoparticles. The proportion of protoplasts stained with FDA and PI was the highest when cells were treated with 360 µM B1CuNPs. As a next step we have tested the effect of B1-Cu bioconjugate on the transformation efficiency of maize protoplasts by cationic polymer.

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P-148

ZIF-8 Zn-MOFs and plant interactions: Size matters in nutritional and toxic effects

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Keywords: nanoparticules , MOFs, colloidal MOFs, ZIF-8, nanoparticles, plant-nanoparticle interaction

The synthesis of MOFs in colloidal form has transformed the development of functional porous materials. Compared to their bulk powder counterparts, colloidal MOF particles offer distinct advantages, including controlled dispersibility, tunable shape, functionalization, transformation, and assembly, enabling a wider range of applications. Among MOFs, the zinc-based zeolitic imidazolate framework-8 (ZIF-8) has emerged as a key material in colloidal science with potential applications in various fields, including agriculture. However, the interactions between ZIF-8 and plants remain largely unexplored. This study examines these interactions using a hydroponic system as a simplified experimental model. Maize plants were exposed to ZIF-8 nanoparticles of different sizes and concentrations to assess their effects on plant growth and nutrient uptake. The results indicate that nanoparticle size had a more pronounced effect on

plant responses than concentration. Furthermore, interactions between ZIF-8 and the hydroponic solution were observed, directly influencing nutrient availability. These findings contribute to a deeper understanding of plant-nanoparticle interactions and provide valuable insights into the potential agricultural applications of ZIF-8.

P-149

Phytotoxicity of two crystalline forms of titanium dioxide nanoparticles

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Keywords: environmental toxicity, anatase, nanoparticle, rutile, wheat

Nano scale particles are receiving increasing attention worldwide due to the properties that might be fundamentally distinct of dissolved materials or microscopic particles for many applications. Nano scale particles are characterized by size ranging from 1 to 100 nm extent at least in one dimension. Nano scale particles of titanium dioxide ($nTiO_2$) are becoming more prevalent in the environment as a consequence of their large-scale industrial utilization. The $nTiO_2$ particles are known in two crystalline form: rutil and anatase, where rutile is a nanoparticle in two dimensions, whereas anatase is in all three dimensions. Although the European Food Safety Authority (EFSA) has updated the safety assessment of titanium dioxide (E 171) in the past years that $nTiO_2$ as a food additive colourant is not considered being safe. In spite, data are scarce on the environmentel effect and phytotoxicity of $nTiO_2$ crystalline forms. This study aims to evaluate the potential phytotoxicity of TiO_2 nanoparticles specifically the anatase and rutile crystalline form, on bread wheat (*Triticum aestivum*). Since accumulation of $nTiO_2$ particles in a high density means an additional risk, we were also aimed to model the effect of accumulation. Manufactured $nTiO_2$ particles (anatase and rutile; NTA-20-S25-01 and NTR-20-02, respectively) were applied on wheat seedlings at three leaves stage in a concentration range from 50 to 3000 ppm. Experiments were performed in hydroponics as short (1-2 days) and long term (14 days) exposure. Physiological responses: status of the photosynthetic apparatus, malondialdehyde (MDA) content, activity of antioxidative enzymes (ascorbate peroxidase activity; catalase) were analyzed together with element mapping by X-ray fluorescence (XRF) imaging. Data emphasize that anatase was nottoxic, while rutile showed toxic effects only at higher concentrations, connected with the increase in the Ti-Kα XFR signal. Importantly, high MDA levels and reduced photosynthetic efficiency were shown under high rutile exposure, pointing to oxidative damage and reduced photosynthetic efficiency. These outcomes illustrate the need to differentiate between nanoparticle types in assessing environmental impacts. Follow-up studies can highlight the ecological impacts of $nTiO_2$ particles through the investigation of long-term exposure effects linked to those nanoparticles, molecular responses, and soil-plant interactions.

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Synthesis of chitosan nanoparticles and its application in agriculture: A review

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Keywords: chitosan, fertilizer, pesticide, controlled released fertilizer

Agriculture is the primary source of food for the global population, and at present there is no alternative that can adequately substitute for it. However, the agricultural sector is faced with a multitude of challenges which may be attributed to biotic (living) or abiotic (non-living) factors. To address these challenges and ensure the productivity of crops, the use of fertilizers, pesticides, and growth enhancers is imperative. Presently, both organic and inorganic agricultural products are available; yet due to the deleterious effects associated with inorganic inputs, there is a growing preference for organic solutions. In this context, chitosan nanoparticles emerge as a compelling, multifaceted organic alternative. Chitosan, a natural substance derived from the shells of marine invertebrates such as crabs and shrimp, has emerged as a prominent solution. As a result of their nanoscale size, these particles are easily absorbed by plants, making them highly effective. Chitosan

nanoparticles exhibit intrinsic antimicrobial properties and function as a natural fertilizer, owing to their richness of nutrients, including carbon, nitrogen, and phosphorus. This makes them a suitable replacement for various conventional fertilizers. Another significant advantage of chitosan is its ability to stimulate enzyme activity and strengthen plant defense mechanisms. Furthermore, the potential of chitosan as a fertilizer coating material is of increasing importance in the context of reducing soil pollution. This objective is accomplished by employing Controlled Release Fertilizer (CRF) technology, a method that facilitates the sustained release of nutrients into the soil. The quality of the resulting product is contingent upon the extraction method employed. Two primary methods are commonly employed: organic and inorganic. Enzymatic treatment and fermentation are two examples of organic methods. Inorganic methods, on the other hand, involve processes such as de-mineralization, de-proteination, and de-acetylation. Following extraction, the next step involves the conversion of chitosan into nanoparticles for agricultural application. Common techniques used for this transformation include ionic gelation and microemulsion.

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P-152

Effect of organic nanofibers carrying auxin on morphology and non-enzymatic antioxidant defense in *Stevia rebaudiana* Bert. under *in vitro* conditions

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Keywords: *Stevia rebaudiana* Bert., *in vitro*, IAA

The interest in antioxidants in foods and dietary supplements has been significant in recent decades. Plant preparations have a strong bioactive potential and play an important role in the detoxification mechanisms of the organism, a factor and stimulant in human health and healing processes. The use of nanoparticles and various phytohormones in plant biotechnology accelerates plant growth and affects the synthesis of a wide range of secondary metabolites. The object of the study is Stevia rebaudiana, a plant with many benefits in the diet of modern man, as the sweetest natural non-caloric substitute for sugar. The hypothesis is that the application of organic nanofibers, carriers of plant growth hormone, will positively affect the physiology, biochemistry and morphology of in vitro plants, thus provoking the formation of pharmacologically valuable antioxidant compounds.

The main biometric indicators of 30-day-old micro plantlets were determined. To quantify the main complexes of biologically active substances in *Stevia rebaudiana* Bert., a number of phytochemical spectrophotometric methods were used to measure the non-enzymatic antioxidant activity. In our study, the application of different concentrations (1 mg L^{-1}, 10 mg L^{-1} and 50 mg L^{-1}) of organic nanofibers (M6) carrying auxin (M6+IAA) in MS medium during micropropagation led to better performance in some biometric parameters and to higher content of most metabolites with antioxidant potential.

From our present investigation, we concluded that the investigated nanofibers influence the *Stevia rebaudiana* Bert. metabolism under *in vitro* conditions and demonstrate a great potential to apply in technologies for obtaining plant tissue cultures. It is a challenge for scientists to seek progress in the development of methodologies involving nanoparticles that contribute to an increased content of substances with antioxidant properties.

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PLANT SECONDARY METABOLISM

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Metabolomic insight into pepper landrace diversity by means of a non-targeted NMR approach

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Keywords: *Capsicum annum*, nutritional quality, metabolomic profile

Pepper (*Capsicum annuum* L.) is an economically valuable crop belonging to the Solanaceae family, widely grown also in the Mediterranean basin. Pepper fruits are well appreciated by consumers and are an excellent source of beneficial compounds as fibers, vitamin C, vitamin E, carotenoids and flavonoids in general, responsible for their health-protective effects. Notably, Italy can be considered a relevant center of diversification, as soil and climate characteristics allow the cultivation of diversified landraces with distinct morpho-agronomical properties and quality-related features. Hence, Italian local varieties have become the focus of multi-omics comprehensive investigations. Exploiting the new developed omics-based tools, indeed, definitely help in gene identification and regulation in plant secondary metabolism.

Recently, several pepper local varieties from the Puglia region (South Italy), some of which at high risk of extinction, were collected. All investigated varieties have diverse morpho-agronomical characteristics, including shape and different color of the fruit. Our research is focused on metabolic and genetic variability of these landraces, with particular attention on quality characteristics of their fruits (BiodiverSO and BiodiverSO Karpos, PSR Puglia 2014-2020, Mis. 10.2 Projects).

Plant metabolomics, combining high-throughput analytical chemistry and multivariate data analysis, represents a reliable and powerful tool to study the complexity of phytochemistry, since it allows to measure and compare simultaneously a pool of metabolites from crude natural extracts. With the aim to valorise local peppers for their peculiar nutritional quality, we used a non-targeted spectroscopic approach combining NMR experiments and multivariate data analysis to provide a comprehensive picture of the chemical composition of 10 pepper landraces from Puglia. Current investigations are also focused on the antioxidant capacity of a subset of the local varieties here analyzed, to better characterize and valorize them, increasing their economical value.

In addition to metabolomic studies, we iniziated whole-genome resequencing analyses of the 21 genotypes collected in Puglia region, representative of the Apulian variability for shape and colour, with the aim to provide new genomic sequences for an in-depth knowledge of the existing diversity, focusing on variations of genes involved in beneficial phenylpropanoid synthesis.

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Novel Members Of The CYP81F enzyme family from *Brassicaceae* plants

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Keywords: CYP81F, glucosinolates, *Arabidopsis*

Glucosinolates are unique ß-thioglucosides produced by plant species belonging to Brassicales order that contribute in plant defense. Depending upon the precursor amino acid, glucosinolates can be categorized into aliphatic, aromatic and indole glucosinolates (IGs) [1]. The core IG biosynthesis leads to indol-3-ylmethyl glucosinolate (I3G), which in turn is modified by monooxygenases of the CYP81F family and the corresponding IG *O*-methyltransferases to form 4-hydroxy-I3G (4OHI3G), 1OHI3G, 4-methoxy-I3G (4MI3G) and 1MI3G respectively. In the model plant *A. thaliana* the *CYP81F* family comprises of four genes. Experimental evidence indicated that CYP81F1, CYP81F2 and CYP81F3 mediates the formation of 4OHI3G, while CYP81F4 functions in biosynthesis of 1OHI3G [1]. Our recent study revealed that species from the *Capsella*, *Camelina* and

Neslia genera, which are closely related with *A. thaliana*, lost the *CYP81F2* and *CYP81F4* genes, but gained two novel *CYP81F* genes with unknown functions named as *CYP81F5* and *CYP81F6* [2].

In our current study, we performed analysis of available genomic sequences of species representing Brassicaceae family to identify *CYP81F* orthologs. We found that genes homologous with *CYP81F5* and *CYP81F6* are also present in a few other not closely linked species of this family. Putative *CYP81F5* orthologs are found in *Thlaspi arvense*, and in two species from the tribe Isatideae. 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. To this end we expressed these enzymes in the *cyp81f2 cyp81f4 A. thaliana* mutant that is deficient in 1MI3G biosynthesis, accumulates strongly reduced amounts of 4OHI3G and 4MI3G in leaves, but hyper-accumulates I3G, which is the substrate of CYP81Fs. Metabolic analysis of the generated transgenic lines indicated *Cr*CYP81F5 is able to hydroxylate I3G to produce 4OHI3G. It has been also concluded that *Cr*CYP81F6 is not capable of modifying I3G.

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Characterization of the metabolic profiling of peach varieties resistant and susceptible to cold-induced damage during storage

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Keywords: *Prunus persica*, metabolomics, chilling injury

Chilling injury is a physiological disorder induced by low temperatures during storage, which may manifest as browning, bleeding, and mealiness. These symptoms significantly affect the postharvest quality and availability of peaches after storage, impacting their global marketability. This study aimed to characterize the metabolic profile of peach varieties with contrasting responses to chilling injury and evaluate their relationship with quality parameters and physiological changes observed at harvest and during storage at different temperatures. Hence, two varieties from the IRTA peach collection were selected based on previous trials: 'Catherine', a variety tolerant to chilling injury, and 'Elegant Lady', a susceptible variety.

Harvested fruit was analysed at three time points: at harvest, after 28 days of storage at two temperatures (0ºC and 4ºC), and after a subsequent shelf-life of 3 days at room temperature (20ºC) for both 0ºC and 4ºC for a total of 5 conditions. At harvest, fruit initial quality was assessed through key quality parameters including: diameter, weight, firmness, chlorophyll absorbance index (I_{AD}), total soluble solids (TSS), and titratable acidity (TTA). After 28 days, and after the 3-day shelf-life, for 0º and 4ºC, key quality parameters were analyzed as well as chilling injury using a three-level scale.

Also, the volatile and metabolomic profiles were analyzed at the 5 conditions using gas chromatography coupled with mass spectrometry (GC-MS), and various protocols for extracting, derivatizing, and detecting metabolic compounds were tested to optimize the analytical conditions. This approach allowed for the detection of a wide range of metabolites, including sugars, amino acids, organic acids, polyols, fatty acids, and other compounds of physiological and biochemical interest.

The study found that 'Catherine' peaches maintained firmness with minimal loss at both temperatures, but showed a significant decrease in titratable acidity in fruits stored at 4°C compared to those stored at 0°C. In contrast, the 'Elegant Lady' peaches experienced a marked loss of firmness, particularly at 4°C, and had a higher incidence of chilling injury, including 100% browning. The other symptoms of chilling injury, bleeding and mealiness, were more prominent in 'Elegant Lady'.

This study provides information on potential biomarkers for predicting the incidence of chilling injury, as well as the basis for its prevention such as the enrichment of certain protective compounds. The findings offer valuable insights for the fruit production sector, aiding in the identification and selection of peach varieties with better resistance to cold storage conditions.

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Establishment of in vitro callus culture of ginkgo (*Ginkgo biloba* L.) for studying the biosynthesis of 3',8''-biflavones

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Keywords: biflavonoids, callus, ginko

In vitro plant culture represents a valuable tool for controlled plant propagation and manipulation under aseptic conditions. Due to its practicality, cost-effectiveness, and speed, it finds broad applications in biotechnology and pharmacy, particularly for obtaining raw materials and bioactive molecules of interest, especially in the context of medicinal plants. One such plant is ginkgo (*Ginkgo biloba* L.), renowned for its various health benefits, including memory enhancement, brain function preservation, and cardiovascular support. These effects are attributed to specialized metabolites, among which 3',8''-biflavones play a significant role. These are flavonoid dimers composed of two monomeric flavonoid units dimerized at the 3'C-8''C position. To date, 13 such 3',8''-biflavones have been identified in ginkgo, with the most prevalent being amentoflavone, bilobetin, ginkgetin, isoginkgetin, and sciadopitysin. These compounds exhibit important biological activities, including antioxidant, antimicrobial, and anticancer properties, making them promising candidates for drug development. However, their specific role in plants, distribution, accumulation patterns, and dynamics remain largely unknown. To address these questions, we established an *in vitro* ginkgo culture, specifically callus culture, which consists of undifferentiated cell masses capable of regenerating whole plants. Callus induction was tested using different ginkgo explants, including leaf blades, petioles, and buds. The best callus formation was observed in leaf blades, followed by petioles, while no callus development occurred in buds. Two methods for callus induction were evaluated, involving different culture media as well as varying auxin and cytokinin combinations and concentrations. Once an optimal and long-term sustainable callus culture was established, the callus tissue was air-dried, extracted with 70% ethanol, and analyzed for 3',8''-biflavones (amentoflavone, bilobetin, ginkgetin, isoginkgetin, and sciadopitysin) using a previously established HPLC-DAD method for their separation and quantification. Preliminary results confirm the presence of four 3',8''-biflavones: bilobetin, ginkgetin, isoginkgetin, and sciadopitysin. Their composition and concentration varied across different samples, depending on the age of the leaves used for callus induction, callus duration, and cultivation method. These findings pave the way for further investigation into the physiological roles of biflavones in plants, particularly in the context of photosynthesis. Moreover, this method will enable future studies on the conditions that promote their accumulation through elicitation strategies.

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Comparison of cyclic hydroxamic acid content in maize (*Zea mays* L.) hybrids

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Keywords: cyclic hydroxamic acids, maize hybrids, hormone treatments

Cyclic hydroxamic acids (cHx) are secondary metabolites produced mainly by members of the *Poaceae* family. The two most abundant cHx in maize are DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) and DIBOA (2,4-dihydroxy-1,4-benzoxazin-3-one). Their production is both constitutive and inductive. Various stress factors can act as inducers. In our experiments, we investigated the cHx content of three maize hybrids (Noa, Armagnac and P9578). Plants were grown in commercial potting soil in the Biodrome greenhouse of the Institute of Applied Plant Biology, University of Debrecen, Hungary, until 2 weeks of age. At 2 weeks of age, the hybrids were divided into 5 groups: control plants and plants treated with mechanical damage were sprayed with distilled water, hormone treatments were applied separately with abscisic acid (20 µmol litre^{-1}), jasmonic acid (100 µmol litre^{-1}) and Na-salicylate (100 µmol litre^{-1}). Mechanical damage was achieved by an equal number of cats on the top leaf. The amounts of DIMBOA and DIBOA were determined 1, 2, 4, 8, 24 and 48 h after the treatments by HPLC using previously isolated standards. We sampled 4 plants per treatment, per sampling date, grown in separate pots. Based on our results, the hybrid Armagnac was found to be the hybrid with the highest content of DIMBOA and DIMBOA+DIBOA, averaged over all treatments and sampling dates. The DIBOA content of the

Noa and Armagnac hybrids was higher than that of the P9578 hybrid, averaged over all treatments and sampling dates. The effect of sampling dates on the average of hybrids and treatments on DIMBOA and DIBOA contents and on the combined amount of these two compounds was significant. The highest cHx content was measured 1 h after the treatments. There was no difference in DIBOA levels at the other sampling times. DIMBOA levels were lower at 4, 24 and 48 hours after treatments compared to levels measured 1 hour after treatments. There was no detectable difference in the cHx content of the Noa and Armagnac hybrids between treatments, averaged over samples. The DIMBOA content of hybrid P9578 averaged over the samples and the combined amount of the two cHx's measured in the control treatment exceeded the DIMBOA and DIMBOA+DIBOA content of the jasmonic and mechanical damage treatments. The effect of stress hormone treatments and mechanical damage associated with the presence of different stress factors is proposed to be elucidated in the future by studying the expression of genes encoding the biosynthesis of cHx-s. This work was supported by the Scientific Publishing Programme of Debrecen University, Hungary and grant EKÖP-24-2-DE-350.

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Light intensity-dependent modulation of biomass accumulation and volatile metabolite profiles in *Valeriana fauriei*

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Keywords: *Valeriana fauriei*, light intensity, biomass productivity, volatile metabolites, medicinal plant cultivation

Light functions dually in plants–as a critical energy source and a complex environmental signal regulating a broad spectrum of physiological and biochemical processes, including secondary metabolite biosynthesis. In this study, we examined the influence of discrete light intensities (100, 400, and 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$) on growth performance, chlorophyll dynamics, and volatile metabolite accumulation in *Valeriana fauriei*, a medicinal plant traditionally utilized for its sedative and neuroprotective properties.

Plants were cultivated for 12 weeks under controlled conditions in a walk-in growth chamber. Morphological assessments demonstrated that lower light intensity (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) sustained greater plant height and chlorophyll content, indicative of delayed photodamage and improved light harvesting efficiency. Conversely, elevated light intensities (400 and 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$) significantly enhanced biomass accumulation, branch proliferation, and root development.

Metabolite profiling using gas chromatography-mass spectrometry (GC-MS) revealed a light-intensity-dependent modulation of key volatile compounds. Specifically, the production of bornyl acetate and isovaleric acid peaked at 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, while valeric acid showed maximal accumulation at 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Our findings highlight that optimizing light intensity is a viable strategy for enhancing the yield and chemical quality of *V. fauriei*. The study provides a scientific basis for the development of precision-controlled cultivation protocols aimed at maximizing both biomass productivity and the biosynthesis of high-value volatile phytochemicals in medicinal plant production systems. Future research will focus on the effects of light quality (spectral composition) to further elucidate the regulatory mechanisms underlying secondary metabolite biosynthesis in *V. fauriei*.

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Stilbene biosynthesis in grapevine: A new perspective on the role of purine-based signaling molecules

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Keywords: dinucleoside polyphosphates, secondary metabolites, signaling molecules

Plant cells utilize dinucleoside polyphosphates (Np_nNs) as signaling molecules that activate defense responses and regulate secondary metabolism. Among these, both purine- and pyrimidine-based Np_nNs have been shown to influence the phenylpropanoid pathway, a key metabolic route for producing protective compounds. Although the involvement of Np_nNs in stilbene biosynthesis has been confirmed in *Vitis vinifera* suspension cell cultures, the

mechanisms underlying this signaling remain largely unexplored.

In this study, we focused on diadenosine triphosphate (Ap_3A) and its chemically modified analogues, $ApCH_2ppA$ and $ApCCl_2ppA$, which exhibit enhanced stability against enzymatic degradation. We assessed their ability to stimulate *trans*-resveratrol production across a 5–500 µM concentration range. *Trans*-resveratrol accumulation peaked at 5 µM, with no further increases observed at higher concentrations.

To better understand the relationship between signal stability and metabolic response, we also examined the enzymatic degradation of Ap_3A in comparison with two chemically modified analogues, $ApCH_2ppA$ and $ApCCl_2ppA$, known for their enhanced resistance to breakdown. As expected, Ap_3A degraded more rapidly than its analogues, suggesting that nucleotide stability may influence the strength and duration of the signaling response. Our findings highlight the important role of Ap_3A in regulating plant secondary metabolism through Np_nN signaling. Additionally, the use of stable analogues has offered further insights into how nucleotide stability can influence biological outcomes. Ongoing studies aim to investigate the impact of Ap_3A and its analogues on the expression of genes in the phenylpropanoid pathway and the transcription of signaling hubs in signal transduction pathways, enhancing our understanding of their role in regulating plant defense mechanisms.

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Accumulation and persistence of phenolic compounds in *Hordeum vulgare* leaves under varying light and temperature conditions

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Keywords: accumulation and persistence of phenolic compounds, *Hordeum vulgare*, light and temperature treatments, secondary metabolism, HPLC-DAD, epidermal flavonoid index

Phenolic compounds (PheCs) are important photo-protective plant secondary metabolites characterized by notable light-screening properties and strong antioxidant activity. Their antioxidant properties, in particular, make them suitable candidates for mediating cross-tolerance responses, as oxidative damage frequently occurs during severe abiotic stresses of diverse origins. Given that PheCs typically persist in plant leaves for several days following an inductive light stimulus, targeted light treatments may effectively prime (pre-acclimate) plant leaves to better tolerate subsequent stress conditions. However, to effectively utilize such priming strategies, it is essential to elucidate the dynamics of PheC synthesis under varying light and temperature conditions, determine how these environmental factors affect their persistence, and ideally, identify the specific sites of PheCs accumulation within plant tissues.

In this study, we summarize results from several experiments investigating the dynamics of PheCs production and persistence in *Hordeum vulgare* leaves under varying conditions of total irradiance, spectral composition, and temperature. The PheCs content was assessed using multiple instrumental approaches: PheCs profiles were analysed by high-performance liquid chromatography with diode-array detection (HPLC-DAD); epidermal flavonoid content was estimated using a Dualex sensor (Force-A, France); and antioxidant activity was estimated by the DPPH assay. Depending on the specific experiment, either primary or secondary leaves were examined. Light and temperature treatments were applied for periods ranging from 7 to 14 days.

Our results clearly demonstrate that the blue-light spectral component is essential for the accumulation of epidermal phenolics and for overall PheCs accumulation under the tested conditions. However, this blue light-induced response requires higher irradiance levels, as its effects under low-light conditions are negligible. Lower temperatures had a generally positive modulatory effect on the total content of soluble PheCs. Moreover, temperature notably influenced the composition of flavonoids, particularly their acylation pattern.

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Integrated multi-omics profiling of warm-water extracts from cereal grains to identify potential bioactive compounds

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Keywords: bioactive compounds, cereal grain, metabolomics, proteomics, warm water extract

Low-molecular-weight proteins (LMWPs) have garnered significant attention due to antioxidant, anti-inflammatory, and antibacterial properties, and their enhanced ability to traverse cell membranes enables targeted health benefits. A recent study demonstrated that warm-water extraction can effectively extract proteins associated with bioactive materials synthesis in crop seeds. Therefore, in this study, we soaked barley, oat, and wheat grains at 55℃ to obtain warm-water extracts (WWEs) and sequentially applied size exclusion filtration using 10KD filter unit to explore the bioactive materials. At first, we performed proteomic analysis using WWEs extracted from each grain, and this approach led to the identification of 570 differentially modulated proteins (DMPs). Further functional annotation of DMPs revealed that these are primarily associated with various cellular metabolic processes such as amino acid metabolism, stress response, and redox regulation. Subsequent metabolomic and peptidomic analyses using LMWPs fractions, enriched by 10KD filter unit from total WWE samples, were conducted to investigate potential bioactive materials in each grain. From these analyses, 747, 2,096, and 366 significant peptides, associated with antioxidant activity, stress response, and amino acid transport, were identified in barley, oat, and wheat grains, respectively. Additionally, we identified a total of 310 metabolites, of which 9 were mainly related to antioxidant, anti-inflammatory, and antibacterial activities with up-regulation exclusively in barley grain. For further verification, cell viability tests were conducted on normal human astrocytes (NHA) and bronchial epithelial cells (16HBE14o). Particularly, we observed that cell viability significantly improved after co-treatment for 72 h with barley LMWPs compared to other extracts. Furthermore, barley LMWPs enhanced the survival rate of 16HBE14o cells when co-treated with the insecticide hydramethylnon (HM). These findings strongly suggest that soaking cereal grains at 55℃ results in the accumulation of diverse proteins and bioactive materials that may help prevent cell damage by regulating the cell cycle during insecticide exposure.

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Postharvest ABA and MeJA treatments enhance antioxidant capacity and phenolic accumulation in blueberry fruits

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Keywords: postharvest, phenolic compounds, antioxidant enzymes, blueberry, plant hormones

Postharvest storage conditions significantly impact fruit quality, particularly the accumulation of bioactive compounds and antioxidant defenses. Plant hormones such as abscisic acid (ABA) and methyl jasmonate (MeJA) play crucial roles in regulating secondary metabolism, affecting phenolic biosynthesis and enzymatic activity. This study evaluates the effects of ABA and MeJA treatments on total phenolic, flavonoid, and anthocyanin contents, and the changed in the gene family of the phenylpropanoid pathway, as well as the capacity of antioxidant enzymes, during the postharvest storage of blueberries.

Biochemical analyses revealed that both ABA and MeJA treatments enhanced the accumulation of bioactive compounds throughout the storage period. Notably, MeJA exhibited a more pronounced effect, particularly on day 5, resulting in higher levels of anthocyanins and flavonoids compared to control fruits. Enzymatic assays demonstrated increased peroxidase (POD) and superoxide dismutase (SOD) activity, with MeJA-treated fruits showing the highest antioxidant response. Gene expression analysis of the phenylpropanoid pathway

confirmed that ABA and MeJA dynamically regulate phenolic metabolism, revealing differential effects between treatments. Specifically, ABA increased the expression of early-stage genes in the pathway, while MeJA predominantly upregulated late-stage genes involved in anthocyanin synthesis. Consequently, ABA induced an early response, whereas MeJA promoted a more sustained increase in phenolic biosynthesis during storage.

These findings suggest that ABA and MeJA can be effective postharvest treatments for enhancing antioxidant capacity and retaining bioactive compounds in blueberries, thereby potentially improving their nutritional quality and shelf life. Future research should investigate the combined effects of these treatments under different storage conditions to optimize fruit preservation strategies

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From discovery to biotechnological application: LCMS-based approaches reveal a constitutive biosynthesis pathway of flavonols in insect pest-resistant soybean genotypes

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Keywords: insect pests, resistance, proteomic analysis, metabolomic analysis

Introduction: Soybean is one of the most important crops in Brazil's economy. However, soybean crops often suffer damage from insect pests, such as *Anticarsia gemmatalis*. Some resistant genotypes were used to decipher resistance mechanisms by evaluating defense compounds, including protease inhibitors (PIs) and flavonoids. This study aimed to evaluate gene expression and metabolic pathways involved in soybean resistance to insect attack.

Methods: Firstly, an LC/MS QqQ-based strategy was applied for broad-range flavonoid profiling of soybean genotypes contrasting in resistance to the pest insect. Product ion and precursor ion scan modes were combined with pseudoMS^3 fragmentation to map flavonoid classes (aglycones) and their glycoconjugates. Secondly, LCMS untargeted analysis was used to discover new metabolites involved in plant resistance. High abundance proteins were depleted by PEG, and 2DE-LCMS was used to map low- abundance dysregulated proteins. Finally, RNM and LCMS^n were used to confirm the structure of deregulated metabolites. Biological activities were confirmed by chemical synthesis and caterpillar survival assays.

Results: The metabolic profiles generated by LC/MS allowed reconstruction of the flavonoid biosynthetic pathways, revealing a constitutive nature of herbivory resistance in the resistant genotype IAC-17 and metabolic regulation for the rechanneling of Quercetin and Kaempferol and their conjugates in soybean. Highest relative abundances were detected for glycosides such as Rutin, Quercetin 3-O-rhamnosylglycoside-7-O-glucoside, and Quercetin-3-O- rhamnopyranosyl-glucopyranoside-rhamnopyranoside in the leaves of the resistant genotype. LCMS untargeted analysis revealed that the methylated form of Rutin, Isorhamnetin Rutinoside, was produced constitutively in the resistant genotypes IAC-17. Likewise, genes encoding flavonol synthase and methyltransferases were highly up-regulated in IAC-17. Overall, some herbivory defense responses appeared to be constitutive characteristics, while others were induced or JA- independent, as verified for flavonol levels. Conversely, salicylic acid levels were higher in IAC-17 and IAC-100 genotypes. Proteins not yet characterized for plant-insect interactions, such as transmembrane receptors and transcription factors, were positively regulated in the resistant genotype IAC-17. LCMS QqQ-based analysis confirmed that constitutive flavonol biosynthesis was inherited genetically from the PI229358 ancestor and maintained in the resistant genotypes IAC-17 and IAC-100. The structure of Isorhamnetin 3-O-Rutinoside was confirmed by RNM and MS^n analysis. The importance of methylation in the resistance mechanism was confirmed by reduced caterpillar survival when fed diets supplemented with molecules of Rutin containing multiple methylations generated by chemical synthesis.

Conclusion: IAC-17 and IAC-100 appear to be genetic sources for studying flavonol biosynthesis and its

relationship with insect resistance. Genetic manipulation of flavonol biosynthesis regulation, driving the production of methylated flavonols, may be a strategy for developing new soybean genotypes showing high levels of resistance to insect attack.

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Molecular characterization of an EMS-induced soybean mutant with low Ab-αg saponin

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Keywords: soybean, saponin, Ab-αg

Saponins are widely distributed as secondary metabolites in the plant kingdom. In soybean, saponins are classified into two main groups: Group A saponins and DDMP saponins. Group A saponins have been suggested to cause bitterness and an astringent taste due to an acetylated second sugar, whereas DDMP saponins lack this sugar moiety. Therefore, the reduction or absence of group A saponins has attracted attention in soybean breeding. In this study, the PE1607 mutant line was isolated from ethyl methanesulfonate (EMS)-induced lines of the soybean cultivar Pungsannamul. This mutant exhibited a reduced level of Ab-αg saponin, the major component of Group A saponins in soybean. Segregation analysis using F_2 lines confirmed that this phenotype is controlled by a single recessive locus. Fine mapping narrowed the candidate region to a 764-kb interval on chromosome 6, within which a candidate gene, *Sg-10*, was identified. This gene harbors a single nucleotide polymorphism (SNP) in the seventh exon (G1307A), resulting in an amino acid substitution from serine to asparagine. Cosegregation analysis using the F_2 population revealed that the low Ab-αg saponin phenotype was tightly linked with the *sg-10* allele. Expression profiling showed that *Sg-10* is highly expressed in developing hypocotyls and cotyledons. To develop *Sg-10* knock-out mutants using CRISPR-Cas9 system, a total of four target sites within the gene were used, with two target sites introduced simultaneously. Further studies will be conducted using these mutants to elucidate the role of the *Sg-10* gene in saponin biosynthesis in soybean.

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P-165

Uncovering the last missing enzyme for pinosylvin biosynthesis in scots pine: characterization of the cinnamic-acid utilizing 4-coumarate:CoA ligase

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Keywords: Scots pine, 4-coumarate:CoA ligase (4CL), pinosylvin biosynthesis, stilbene

Scots pine (*Pinus sylvestris* L.), one of the most economically important tree species in boreal forests, accumulates stilbenes in the form of pinosylvin and its monomethyl ether. They are derivates of cinnamic acid, but the enzyme that catalyses the reaction from cinnamic acid into the intermediate cinnamoyl-CoA, the substrate of stilbene synthase, has been unknown. The typical enzyme on such position in the pathway is 4-coumarate:CoA ligase (4CL), which activates hydroxycinnamates with varying efficiencies, but this enzyme is generally inactive with cinnamate and only few exceptions are known. The objective of this study is to discover the enzyme that catalyses ligation of cinnamic acid to cinnamoyl-CoA in the Scots pine stilbene biosynthesis pathway. Our results showed that four isoforms of 4CL are expressed in Scots pine and one isoform, namely Ps4CL2, exhibits low Michaelis-Menten constant (Km) and high specificity constant (Kcat/Km) for cinnamic acid, therefore indicating its high affinity and preference for this substrate. Furthermore, we discovered that the cinnamate-activating properties of Ps4CL2 are not governed solely by the substrate binding region of the enzyme but instead is a collective contribution of all the amino acids within the enzyme. These findings complete the final missing enzyme in an important decay and disease resistance pathway in Scots pine.

P-166

Phenolics and cell wall remodelling in fruits of tomato overexpressing *GGP1* gene

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Keywords: arabinogalactans, ascorbate, GDP-L-galactose phosphorylase, phenolic compounds, monolignols, red ripe tomato fruit, ripening

Tomato fruit ripening involves complex physiological processes, including the biosynthesis and degradation of cell wall components such as polysaccharides, phenolics, and proteoglycans, leading to fruit softening. In this study, we investigated how the overexpression of *GDP-L-galactose phosphorylase* (*GGP1*) – a key gene in the L-galactose-dependent ascorbate (Asc) biosynthesis pathway - under the control of two fruit-specific promoters, namely *PPC - phosphoenolpyruvate carboxylase* and *PG – polygalacturonase,* influences cell wall properties and phenolic profile. It was shown that *GGP1* overexpression increases Asc content in ripening tomato fruit and upregulates genes associated with cell wall remodelling [1]. Here, we demonstrated that *PPC-GGP1* and *PG-GGP1* transgenic lines exhibited significant structural alterations in the fruit parenchyma compared to wild-type fruits, particularly a disruption of cell wall composition and organisation [2]. These included modifications in spatial distribution and chemical composition of homogalacturonans and arabinogalactans, as well as changes in the pectin methylation degree. Alongside with reduced content of free chalconaringenin, *p*-coumaric and protocatechuic acids, the amount of cell wall-bound *p*-coumaric acid and the corresponding monolignol, *p*-coumaryl alcohol, was higher in the transgenic fruits. These findings highlight a metabolic interplay between Asc biosynthesis and cell wall remodelling, with potential implications for post-harvest fruit quality and storage.

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P-167

Functional and biosynthetic investigation of long-chain polyisoprenoids of *Rosa chinensis* leaves

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Keywords: *Rosa chinensis*, polyisoprenoids, *cis*-prenyltransferase

Roses (*Rosa* spp.) are well-characterized for their monoterpenoid biosynthesis pathways due to their highly valuated in the fragrance industry. However, little is known about other classes of isoprenoids produced by roses, particularly polyprenols. These compounds, defined by the general formula H-$(C_5H_8)_{n-1}$-C_5H_{10}-OH, are synthesized through the polymerization of isoprene units and vary in length from short to very long chains, including natural rubber. Polyisoprenoids occur in all living organisms, either as single molecular species or as mixture(s) ('families') with one predominant alcohol. In *Rosa* spp., long-chain polyisoprenoids, reaching up to 45 isoprene units, were found to accumulate in leaf tissue in exceptionally high quantities. The biosynthetic origin and physiological function of these long-chain polyisoprenoids in roses remain poorly understood.

Chain elongation of polyisoprenoids is catalyzed by *cis*-prenyltransferases (CPTs), enzymes that determine the final length of the polyisoprenoid product. In *Rosa chinensis* leaves three potential CPTs and one CPT-binding (CPTB) were identified. The subcellular localization of RcCPTs and RcCPTB, as well as their products, was determined through transient transformation of tobacco. CPT2 was localized in the chloroplasts, while the CPT1, CPT3 and CPTB were found in the endoplasmic reticulum. Interestingly, the

chromatographic analyses of *R. chinensis* leaf extracts revealed a wider spectrum of polyisoprenoid than could be explained by the activity of the above mentioned RcCPTs alone. These findings suggest that additional factors, beyond CPTs itself, may modulate the CPT activity and influence the chain-length termination of rose polyisoprenoids. The role of these long polyisoprenoids was also explored. While plastidial polyisoprenoids in *Arabidopsis thaliana* are implicated in high temperature stress responses, our data do not support a similar role in roses. This suggests that their function may be more diverse and warrants further investigation.

This study provides the first molecular insights into CPTs in roses, highlighting a unique diversity of polyisoprenoid products in *R. chinensis*. Our findings establish a foundation for future research on the enzymatic mechanisms and biological functions of long-chain isoprenoids in plants.

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PLANT-PLANT INTERACTIONS AND BIOCONTROL

P-168

Plant responses to pollinator vibroacoustic signals: Effects on nectar and volatiles in *Antirrhinum litigiosum*

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Keywords: vibroacoustic signaling, plant responses, insect-plant communication

Plants can detect and respond to environmental stimuli, ranging from changes in light and temperature to more subtle changes such as volatiles or mechanical touch. However, the capacity of plants detecting and responding to vibroacoustic signals has not been studied in such detail until recent years. Research has shown that plants exposed to predator vibrations activate stronger defense mechanisms and that *Oenothera drummondii* increases nectar sugar concentration in response to the vibration of its pollinators. Under the framework of the Good Vibes project, funded by the Human Frontiers in Science Program, we further examined possible plant responses to acoustic stimuli. We treated *Antirrhinum litigiosum* plants grown in semi field conditions with a playback of its most efficient pollinator (*Rhodantidium sticticum*), pinknoise and silence for 3 hours. Nectar was extracted for sugar concentration measurements and flowers were collected for volatile analysis, immediately after the first treatment and following five consecutive days of daily 1 hour treatments. Results show that both the concentration and the total amount of glucose, fructose and sucrose are significantly higher in plants treated with the playback specially after 5 days of treatment, whereas the nectar volume only experienced a significant increase on day 0. Additionally, transcriptomic analyses were conducted on plants grown

in a controlled climatic chamber to examine gene expression responses in both flowers and leaves associated with nectar production and volatile emission. These results contribute to the growing body of evidence that plants can perceive and respond to sound cues, highlighting a potential plant-pollinator interaction.

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P-169

Genetic and molecular basis of the plant response to competition in *Arabidopsis thaliana*

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Keywords: plant-plant interactions, competition, transcriptomics

Throughout their life cycle, plants interact with many other plants, thereby forming complex networks of interactions that influence the functioning of plant communities as well as crop yield. However, despite the recognized importance of plant-plant interactions, the genetic and molecular basis underlying the natural variation of such interactions remain largely unknown, in contrast to other types of biotic interactions (Subrahmaniam et al., 2018). To address this gap, we integrate approaches from quantitative genetics, phenomics and functional genomics to gain knowledge on the mechanisms underlying plant-plant interaction with a focus on plant competition.

We conducted a Genome-Wide Association (GWA) mapping on natural genotypes of *Arabidopsis thaliana* to identify QTLs controlling natural variation in the response to competition against several species of weeds (i.e. *Poa annua*, *Stellaria media* and *Veronica arvensis*). We observed that QTLs were highly dependent on the identity of the competing species, as well as on the composition of the assemblages, underscoring a high degree of biotic specialization in plant-plant interactions.

This work was complemented by an RNA-seq experiment performed on leaves and roots of *A. thaliana* to obtain a comprehensive overview of the signaling pathways modulated at different stages of interaction with the same weed species. Corroborating the GWA mapping, transcriptional changes in *A. thaliana* in response to competition - as well as their dynamics - are highly specific to the type of competitor. However, we also identified common signaling pathways that are enriched regardless of the competitive conditions

These results, along with the cloning and functional validation of a gene underlying the competitive response of *A. thaliana*, will be presented to provide a comprehensive overview of the genetic and molecular bases involved in plant competition.

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P-170

Antimicrobial potential and phytochemical characterization of *Peucedanum oreoselinum*

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Keywords: *Peucedanum oreoselinum*, antimicrobial activity, bioactive compounds, phytochemical analysis, plant secondary metabolites

Wild plant species are of significant ecological importance and are increasingly recognized for their reservoir of bioactive secondary metabolites. In this study, we investigated *Peucedanum oreoselinum*, a perennial herbaceous species belonging to the Apiaceae family. *P. oreoselinum* has historically been used in traditional medicine for its diuretic and antispasmodic properties. However, there are only a few studies on this plant species so far.

Here, we evaluated extracts from different plant organs for their antimicrobial activity. Ethanolic extracts of *P. oreoselinum* were tested *in vitro* using bioassays against the soilborne phytopathogen *Rhizoctonia solani*. The strongest antimicrobial effects were observed in the seed and root extracts.

To identify the bioactive compounds responsible for the observed antimicrobial activity, crude plant extracts were initially subjected to solid phase extraction (SPE), followed by preparative high-performance liquid chromatography (HPLC) to achieve further fractionation and enrichment. The resulting fractions were subsequently tested for antimicrobial activity using bioassays. Fractions exhibiting significant activity were selected for detailed chemical analysis.

Structural elucidation of the antimicrobial compounds was carried out using nuclear magnetic resonance (NMR) spectroscopy in combination with mass spectrometry (MS). These analyses led to the identification of six compounds with confirmed antimicrobial activity. Among these, one compound was classified as a polyacetylene, while the remaining five were identified as angular angelicin-type furanocoumarin compounds. Two of these furanocoumarin compounds matched previously described structures based on spectral comparison with established databases. However, three furanocoumarin compounds did not match any known entries, indicating that they represent novel antimicrobial compounds. Consequently, three previously undescribed furanocoumarin compounds with antimicrobial activity were identified in this study.

To further investigate the spatial distribution of the bioactive compounds within plant tissues, matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry was employed. This technique enabled the visualization of compound localization in root tissues, providing valuable insight into their biosynthetic origin and potential ecological roles.

P-171

Optimizing cropping practices towards increased photosynthesis efficiency

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Keywords: plant density, land use efficiency, maize

Photosynthesis is a crucial process through which plants convert light energy into chemical energy. This process is essential not only for plant growth but also for the overall health of ecosystems and agriculture. The objective of this study is to examine how maize plants interact with one another in relation to their spacing, optimize photosynthetic efficiency through field trials, and identify the most effective planting density. Six maize hybrids (H1-H6) from the maturity group FAO 400 were cultivated at six different planting densities, ranging from 40,000 to 90,000 plants per hectare (D1-D6), to assess various traits: leaf mass to ear (LME), leaf dry mass to ear (DLME), chlorophyll content (CHLO), leaf area to ear (LAE), husk area (LAH), husk mass (HM), and husk dry mass (DHM). The experiment was conducted at the Zemun Polje during the 2023-2024 growth season. Data analysis revealed that D1 density had the highest values for all leaf-related traits, while D2 density did not produce significant results for the measured traits. Among the evaluated hybrids, H6 excelled in four traits–DLME, LAH, HM, and DHM–when tested at the D1 density. Significant changes in LAE, LAH, LME, DLME, and HM were observed at higher planting densities, particularly at D2 and D3. Notably, significant differences in chlorophyll content were also evident between the first two densities. Moreover, the differences in mean values of morphological traits across various genotypes were especially pronounced for four traits: DLME, CHLO, LAE, and LAH. These findings underscore the complex interactions within cropping systems, particularly the relationship between vegetative area, leaf area, spatial distribution, and genetic potential. Collectively, these factors determine the optimal planting density for maize cultivation. Adjusting planting density can enhance photosynthetic efficiency and better use natural resources.

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P-172

The application of the yeast *Rhodotorula mucilaginosa* enhances maize growth under both drought and well-watered conditions

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Keywords: antioxidant enzymes, drought, stress markers, yeast

Biostimulants offer a sustainable solution to drought stress in plants. Yeasts have received relatively little attention in the biostimulant research even though they have potential to be beneficial for plant growth. These microorganisms might be effective in plant-growth promotion, due to the production of beneficial metabolites such as phytohormones, amino acids, nutrients and enzymes. Mentioned substances, in turn, enhance plant growth, root development and stress tolerance. Yeasts are commonly found in soil; and are members of the microbiome inhabiting above- and below-ground parts of different plant species. In addition, the microbial activity of yeast improves soil health by promoting nutrient availability and establishing microbial communities. This study examined the effects of *Rhodotorula mucilaginosa* (CCY 20-1-39, CCY – Culture Collection of Yeasts at the Institute of Chemistry, Slovak Academy of Sciences) on maize (*Zea mays* L.) under well-watered and drought conditions. Yeast suspensions were applied to grains. After germination, the grains were planted and plants grew for 21 days in controlled conditions in growth chamber with 16/8 h (day/night) photoperiod, at 24 °C/20 °C, and with a humidity level of 45%. Drought reduced shoot length, biomass, and leaf area, but yeast treatment (10^8 cells ml^{-1}) improved these traits. It enhanced relative water content, reduced oxidative stress by lowering hydrogen peroxide and malondialdehyde levels, and increased antioxidant enzyme activity (catalase, guaiacol peroxidase). Decreased oxidative stress and enhanced biomass of roots and leaves resulted in the higher concentrations of photosynthetic pigments, which in turn could potentially increase the assimilation of carbon and biomass building. Under well-watered conditions, yeast also boosted shoot biomass and chlorophyll content. These findings highlight *R. mucilaginosa* as a promising biostimulant for climate-smart agriculture, enhancing maize growth and drought resilience.

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P-173

Seed germination, resistance to stress, and exudation of pea landraces

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Keywords: osmotic stress, Pisum, phenolics

Seed germination is a crucial developmental stage that determines successful seedling establishment. The process is initiated by the uptake of water during imbibition, which reactivates metabolic pathways, and it is highly sensitive to environmental conditions, particularly water availability. To investigate natural variation in germination performance under water-limited conditions, we assessed 117 pea (*Pisum sativum*) landraces, the locally adapted genotypes. Seeds were germinated under control conditions (water) and under osmotic stress induced by polyethylene glycol (PEG 6000). Germination traits were scored and landraces were grouped into three distinct clusters, reflecting varying levels of stress tolerance. Notably, 33 landraces exhibited significantly higher germination rates and final germination percentages under stress, suggesting enhanced physiological resilience to water limitation. Furthermore, we observed a negative correlation between seed size and stress tolerance, with larger seeds generally more susceptible to PEG inhibition, indicating a potential trade-off between seed size and desiccation tolerance mechanisms. In addition, we examined the metabolite and protein composition of compounds released by germinating seed into the surroundings. Proteomic analysis revealed that oxidoreductases

(notably peroxidases), glycosidases, and various hydrolases were the predominant protein classes present in the exudates, suggesting active remodelling of seed cell walls and detoxification of reactive oxygen species. Metabolomic profiling showed that exudate from modern pea genotypes was enriched in simple phenolic acids (particularly caffeic acid). On the other hand, landraces secreted a more diverse array of polyphenolic compounds, including epicatechin, epigallocatechin, or naringenin. These metabolites are known to play roles in antioxidative defense, allelopathy, and signalling, and their elevated presence in landrace exudates may reflect adaptation to heterogeneous environments. In summary, our findings highlight substantial natural variation in seed germination capacity under osmotic stress and uncover biochemical differences in seed exudation patterns between landraces and modern cultivars.

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P-174

Changes in differential emissions of biogenic vocs, reactive aldehydes and metabolites under heat and root anoxia in winter wheat

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Keywords: biogenic volatile organic compound, reactive carbonyl species, secondary metabolites, wheat

Biogenic volatile organic compounds (BVOCs) from plants are extremely diverse. The odour of the plants are one of the best known classes, the terpenes of BVOCs. There are several other organic compounds that have a high vapour pressure at room temperature. Stress factors also affect BVOC emissions from plants.

Reactive carbonyl species (RCS), including aldehydes, due to the high reactivity of these compounds, which are often present in the form of covalent adducts with DNA, proteins or other biomolecules extremely challenging for detection and quantification.

Secondary metabolites are important for plants because they provide information about the current state of the plant. It is well known, that the changes in the different sugar components in the case of cold stress show how the plant is prepared to prevent cold damage. The TCA cycle is important in plant for respiration, energy release and precursors product for amino acids. Amino acids also accumulate under stress. Changes in secondary metabolites provide a lot of physiological information.

Our study focuses on the free fraction of the BVOCs under different abiotic stress conditions (heat, flooding, their combination) in winter wheat. Plants emit BVOCs from the leaves, so the free forms of the compounds should measure this fraction. We measured the extracted compound using triple-quad GC-MS coupled with SPME technique. The SPME fiber was PDMS, the carrier gas was He and the thermal program was started at 40°C and ended at 250°C. The free forms of the secondary metabolites are measured after derivatised with N-trimethylsilyl-N-methyltrifluoroacetamide with LECO 4D GCxGC TOF-MS (LECO Corp.) was used in split mode in targeted analyzing mode.

The analysis revealed a large number of BVOCs, including acetaldehyde, propionaldehyde, isobutyraldehyde, n-butanal, methyl vinyl ketone, 2,4-hexadienal, n-hexanal, n-heptanal, pentenal, octanal, nonanal, and 1-penten-3-ol, cis-2-penten-1-ol, 7-octen-4-ol, 1-octen-5-ol, cyclohexene, tetrahydrolinalool, which showed significant changes under heat and flood stress. The measurement of the different sugar contents, like glucose, fructose, and the TCA cycle components are very important.

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MusaNAC46 transcription factor regulates stress induced leaf senescence of banana plant by modulating autophagy and chlorophyll catabolism

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Keywords: stress, senescence, autophagy, chlorophyll catabolism, abscisic acid

Stress induced senescence negatively impacts crop productivity in plants and also plays a crucial role in their growth and development. Stress-related NAC transcription factors are important molecular mediators of senescence in model plants such as Arabidopsis, but

their mode of action is completely unknown in important crop plants. In this study, mechanistic role of the NAC transcription factor 'MusaNAC46' was elucidated in banana plants. Transcriptional analysis showed the MusaNAC46 transcript to be upregulated under salt as well as drought stress. Notably, overexpression of MusaNAC46 (MusaNAC46-OX) led to precocious senescence phenotype in banana plants, whereas the knockout (MusaNAC46-KO) lines exhibited delayed senescence phenotype, highlighting its function in the regulation of senescence in this plant. Leaves of MusaNAC46-OX lines showed a considerable reduction in the chlorophyll content as compared to control, whereas MusaNAC46-KO lines displayed the reverse, i.e. the chlorophyll levels were enhanced. Transmission electron microscopy revealed the presence of degraded chloroplasts and autophagic bodies in the leaf tissue of MusaNAC46-OX lines. Transcript abundance analysis showed upregulation of two key chlorophyll catabolic genes, *nonyellow colouring (NYC)* and *pheophytinase (PPH)*, along with the upregulation of several autophagy-related genes (e.g. *ATG5, ATG8B*, and *ATG12*). In contrast, expression of these genes in knockout lines was down regulated. A dual luciferase assay confirmed the direct interaction of MusaNAC46 transcription factor to the promoters of *NYC, PPH, ATG5, ATG8B,* and *ATG12*. LC-MS analysis demonstrated a notable increase in ABA levels and enhanced accumulation of H_2O_2 in the leaves of the MusaNAC46-OX lines, which points to the involvement of MusaNAC46 in stress-induced senescence. In conclusion, the results obtained from our work show that levels of MusaNAC46-like proteins may be modulated to modify senescence response in plants.

P-176

Immunolocalization of aquaporins in the leaves of two *Solanum tuberosum* L. genotypes differentiated in terms of sensitivity to drought

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Keywords: aquaporins, drought stress, potato, transmission electron microscope

In the last decade, the frequency of long-term drought periods in Central Europe has increased, which has serious consequences for the yield of crops, including potato (*Solanum tuberosum* L.). The plant aquaporins from the PIP subfamily (plasma membrane intrinsic proteins) are widely studied in the context of their key role in the regulation of the cell water potential under stress conditions.

In this work, we present the changes in the intensity of labelling of aquaporins in stressed plants of two potato varieties differing in tolerance to drought stress: Gwiazda - resistant genotype to soil drought and Oberon – susceptible genotype to water deficiency. For immunolocalization of aquaporins, leaf samples were prepared according to standard procedures in two experimental variants: 1) control (watered plants) and 2) drought (unwatered plants). The primary antibody specific to PIPs and secondary antibodies conjugated to 10 nm gold particles were used to visualize aquaporins under a transmission electron microscope (TEM).

The observed changes in aquaporin labeling in the membrane of the palisade and spongy mesophyll in one of the tested potato cultivars under drought stress may indicate the ability of this genotype to maintain proper water balance and physicochemical activity under conditions of water deficiency.

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Differences in physiological and morphological parameters of two potato cultivars differing in tolerance to soil drought

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Keywords: potato, drought, heat, physiological and anatomical parameters

The experiment involved analysis of selected morphological, physiological and anatomical parameters related to the photosynthetic efficiency of plants in order to assess the level of tolerance to stress: drought and high temperature in the tested potato genotypes.

The study was conducted on 2 potato varieties with extreme tolerance to soil drought. Denar –resistant genotype to soil drought and Lenka – sensitive genotype to water shortages. After growing in optimal conditions during the tuberization period, the plants were subjected to soil drought and high temperature stress in 4 experimental variants: (1) watered plants, growing in optimal temperature conditions (21°C, control), (2) unwatered plants, growing in optimal temperature conditions (21°C, drought), (3) watered plants and subjected to high temperature (38°C) and (4) unwatered plants and subjected to high temperature (38°C, drought).

Among the morphological and physiological parameters: the ratio of leaves to stems, the assimilation area, the mass of leaves, the mass of stems, the greenness of leaves SPAD, the rate of wilting was assessed. The parameters of the quantity and quality of the yield were also assessed.

The analysis of the anatomical structure concerned 4 parameters such as: leaf thickness, the epiderma thickness, the palisade and spongy mesophyll thickness. In order to prepare microscopic preparations with cross-sections of leaves, the "freezing" technique and a cryostat type device were used. Microscopic observations were carried out in a light microscope (Olympus).

The analysis of microscopic images showed that in the case of Denar cultivar plants, drought stress did not significantly affect the values of the anatomical parameters studied, only in the variants where high temperature was applied (variant 3) and the combination of drought and high temperature a slight decrease was noted for the leaf thickness in variant 4 and the spongy mesophyll thickness in variants 3 and 4. On the other hand, in the leaves of Lenka cultivar, under stress conditions (variants 2-4), a clear decrease was observed for the values of leaf thickness and epiderma thickness, as well as palisade and spongy mesophyll thickness compared to the control plants.

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Genetic background of the hairy phenotype of two cbf transgenic barley genotypes

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Keywords: CBF, cereals, trichome

It was proved earlier, that CBF14 and CBF15 transcription factors have outstanding role in the development of frost tolerance in wheat (Soltész et al., 2013). The genes encoding these transcription factors were isolated from winter wheat (*Triticum aestivum* L. ssp. *aestivum* cv. 'Cheyenne') then over-expressed in spring barley (*Hordeum vulgare* L. cv. 'Golden Promise'). The RNA-seq based transcriptome analysis revealed, that hundreds of genes changed their expression as a result of transgenes. Beyond the enhanced freezing tolerance, an interest hairy phenotype was observed on the leaves of transgenic barleys. The structure of the trichome was analysed by electron microscopy and the genetic background of this development was investigated by in silico bioinformatics methods. Based on the Gene Ontology annotations and TAIR (The Arabidopsis Information Resources) database, we have found about 75 trichome associated, differentially expressed genes. Furthermore, a promoter analysis was performed for screening the suspected cis-acting elements which might

be regulates the cold and dehydration-responsive, trichome associated gene expression.

This work was supported by the National Research, Development and Innovation Office (NKFIH) STARTING-149613 and TKP2021-NKTA-06 grants.

Soltész, A., Smedley, M., Vashegyi, I., Galiba, G., Harwood, W., & Vágújfalvi, A. (2013). Transgenic barley lines prove the involvement of TaCBF14 and TaCBF15 in the cold acclimation process and in frost tolerance. Journal of Experimental Botany, 64(7), 1849–1862. https://doi.org/10.1093/jxb/ert050

P-179

Priming with putrescine and oximes alleviate the impact of drought stress on *Medicago truncatula*

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Keywords: drought, oximes, polyamines

Drought is currently one of the most significant limiting factors in production. Their importance is rising due to the increase in climate alterations such as reduced rainfall or extreme weather events. Therefore, the understanding of plant tolerance to drought stress has become an urgent matter. In this context, the root system architecture is gaining interest as one of the most influent adaptation. In fact, several root traits, as the stimulation of the lateral root growth, have been found to increase productivity under drought. In recent years, various pieces of evidence suggest that polyamines and oximes play a role in the development of the root system, potentially mediated by the activation of nitric oxide synthesis (Urra et al., 2022; López-Gomez et al., 2024). The present project aims to evaluate the impact of priming treatments with the polyamine putrescine (Put) and the oxime indole-3-acetaldoxime (IAOx) in the alleviation of drought stress in *Medicago truncatula* plants. Seeds of *Medicago truncatula* Gaertn ecotype Jemalong A17 were germinated on Petri dishes containing 0.4% (w/v) plant agar for 72 h at 14 °C in darkness. Sprouts were transferred to glass jars under axenic conditions, which contained 100 ml of modified Fahraeus media with 3 g l^{-1} of phytagel growth medium. A set of plants was primed with IAOx at 100 M, a second set with Put at 500 M and a third set was maintained without any priming as control, for a growth period of 6 days. Then, a plant subset was harvested, shoots and roots were separated, and biomass production as dry weight was measured. Tissue aliquots were frozen in liquid nitrogen, and stored at –80 °C for further analyses. Another set of plantlets of each priming treatment was transferred to 0.2 L pots with vermiculite:perlite (2:1) and the drought treatment was applied for 10-15 days until the substrate water availability was reduced to 50-75% of the field capacity, in parallel to well irrigated pots at 100% field capacity. Preliminary results show that the priming with Put and IAOx to plantlets positively impact on root development under water deficit conditions. Through the analysis of genes and metabolites involved in Put and IAOX metabolism, we aim to interpret the mode of action of each compound on the development of the root system under water stress conditions.

Urra et al. (2022). J. Exp. Bot. 73: 5581–5595.

López-Gómez et al. (2024) Molecular Plant 17, 178–198.

P-180

Comprehensive DIA-MS approach of rice basal nodes elucidates mechanisms of salinity tolerance

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Keywords: Oryza sativa, root basal node, salinity stress, DIA-MS, proteomics

Global climate change and environmental pollution have imposed severe challenges on global food security by exacerbating abiotic stresses such as salinity. In response, recent advances in molecular breeding and genetic engineering have facilitated the development of multi-stress-tolerant rice varieties. A notable example is the successful introgression of Submergence 1 (Sub1),

Anaerobic Germination 1 (AG1), and Pi9 QTLs into Ciherang, a widely cultivated Indonesian rice variety, resulting in the CSA-Pi9 line exhibiting improved tolerance to submergence, salinity, and blast disease. In this study, we conducted a comprehensive proteomic investigation to elucidate the molecular basis of salinity tolerance using data-independent acquisition mass spectrometry (DIA-MS). Basal node tissues were collected from Dongjin (DJ), CSA, and CSA-Pi9 rice varieties following salinity stress treatment. A total of 9,350 proteins were identified, among which 3,016 were differentially modulated in response to salinity stress. Hierarchical clustering grouped these proteins into seven distinct clusters, reflecting diverse regulatory patterns across genotypes. Notably, 234 and 275 proteins were specifically upregulated in the CSA and CSA-Pi9 varieties, respectively. Functional enrichment analysis revealed that these proteins are predominantly involved in key metabolic pathways, including the tricarboxylic acid (TCA) cycle (e.g., pyruvate dehydrogenase, citrate synthase, succinyl-CoA ligase, and malate dehydrogenase), mitochondrial electron transport (ATP biosynthesis), ion transport (Na^+/K^+ channels and H^+-ATPases), cellular signaling, structural organization, and protein biosynthesis. Collectively, our findings provide proteome-wide insights into the complex regulatory networks that underlie salinity tolerance in rice, offering potential targets for future crop improvement strategies

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Apple responses to frost are affected by inoculations with cold-tolerant bacteria

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Keywords: frost stress, cold-tolerant bacterial endophytes, apple plants

Climate warming is responsible for mild winters and warm springs, causing premature plant development and increasing the risk of exposure of vulnerable plant tissues to spring frost. Cold stress triggers changes in membrane fluidity and reactive oxygen species (ROS) levels, causing damage to plant tissues. Wild plants growing in alpine regions are associated with complex microbial communities that may support plant growth and survival under cold conditions. However, limited information is available on the beneficial plant-microbe interactions in the mitigation of freezing stress. The aim of this project was to characterize the efficacy and mode of action of cold-tolerant endophytic bacteria isolated from alpine Rosaceae plants in mitigating freezing stress in apple plants. Three cold-tolerant bacteria, belonging to *Pseudomonas* and *Duganella* genera, were selected according to their ability to decrease electrolyte leakage in freezing-stressed strawberry seedlings. Apple seedlings (cultivar Golden Delicious) were grown under controlled conditions, treated (bacterium-inoculated) or not (mock-inoculated) with cold-tolerant bacteria, and exposed to frost simulation. Preliminary results showed that bacterial inoculation decreased ROS content in leaf tissues after freezing stress. Transcriptomic analysis revealed the upregulation of genes related to gene ontology (GO) biological processes of carbohydrate metabolism, stress response, and signal transduction in mock-inoculated and bacterium-inoculated plants after freezing stress. Moreover, plants inoculated with cold-tolerant bacteria upregulated the expression of genes related to nucleotide metabolism and cell cycle. These findings highlighted the potential contribution of cold-tolerant bacteria isolated from alpine Rosaceae plants in enhancing apple resilience to freezing stress and created the basis to improve agricultural sustainability under climate change.

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P-182

A multi-layered approach to dissect photosynthetic and molecular adaptation of plants to increasing urban CO_2 levels

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Keywords: CO_2 stress, plant adaptation, urban ecology

Urban ecosystems, though limited in area, are hotspots of abiotic stress due to high CO_2 emissions, elevated temperatures, pollution, and altered hydrogeological cycle. Among these, CO_2 enrichment represents a persistent and often overlooked abiotic stress that shapes plant physiology and evolution. Our research aims to investigate how prolonged exposure to urban CO_2 levels influences plant responses at physiological, cellular, and molecular levels. Using *Populus alba*, we conduct comparative analyses between individuals growing in urban Florence and nearby non-urban green areas. We assess RuBisCO enzymatic activity through high-throughput assays and examine changes in chloroplast structure, leaf cuticle, and photosynthetic tissues via confocal and transmission electron microscopy. Data were then integrated with determination of enzymatic activity related to key enzymes (CAT, APX, SOD, peroxiredoxin, and glutathione reductase), to evaluate the redox state alteration due to altered CO_2 levels. Chloroplast genomes are then analyzed to uncover potential structural rearrangements, mutations, and gene content changes associated with long-term CO_2 exposure. To complement these site-specific insights, we are performing a in-deep transcriptomic meta-analysis of *Arabidopsis thaliana*, *Populus*, and other plant species from publicly available datasets focused on CO_2 stress. This approach allows us to identify differentially expressed genes (DEGs), co-expression modules linked to CO_2 responses, organ-specific expression signatures, and novel candidate genes potentially involved in abiotic stress tolerance mechanisms. RNA-seq data were also coupled with metabarcoding analyses of fungal and bacterial endophytic communities to deepen the plant stress response from a holobiont perspective. This work was supported by the PRIN grant (MUR, 2022RYTHE3, Italy) and contributes to the identification of key stress-response markers and adaptive traits, supporting the selection of resilient species for urban environments and climate-adaptive planting strategies.

P-183

An improved nondestructive method to detect plant heavy metal toxicity on a fine timescale

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Keywords: electrical capacitance, dissipation factor, root conductance, transpiration, water uptake

Toxic accumulation of heavy metals in soils due to human activities such as urban traffic, mining, industrial waste, sewage sludge, phosphate fertilizers or agrochemicals, has become a global problem. The root dielectric response was measured on a minute scale to assess its effectiveness in non-destructively monitoring short-term cadmium (Cd) toxicity. The time-series methodology required the design and construction of a specialized electrical measurement system. Electrical capacitance, dissipation factor and electrical conductance were detected in potted maize, cucumber and pea from 24 to 168 hours after Cd treatment (0, 20, 50 mg Cd^{2+} kg^{-1} substrate). Stress was also assessed by measuring leaf chlorophyll content, F_v/F_m and stomatal conductance *in situ*, and shoot and root mass and total root length after harvest. Root electrical capacitance showed a clear diurnal pattern, reflecting water uptake rate, and decreased significantly in response to excessive Cd due to impeded root growth, reduced tissue permittivity caused by accelerated lignification, and root ageing. Cd exposure markedly increased the measured dissipation factor, indicating greater conductive energy loss due to oxidative membrane damage and increased electrolyte leakage. Electrical conductance, which was coupled to

root hydraulic conductance and varied diurnally, was transiently increased by Cd toxicity due to enhanced membrane permeability, but subsequently decreased due to stress-induced leaf senescence and transpiration loss. The time series of the impedance components indicated the comparatively high Cd tolerance of the applied maize and the sensitivity of the pea cultivar, which was confirmed by visible shoot symptoms, repeated physiological investigations and biomass measurements. The results demonstrated the potential of single-frequency dielectric measurements to monitor certain aspects of the stress response of different species on a fine time scale without causing plant damage. The approach can be combined with widely used plant physiological methods and could contribute to the breeding of crop genotypes with improved stress tolerance. In addition, the dielectric assessment of tolerance and acclimation responses to heavy metal pollution may help to evaluate the phytoremediation potential of plants, contributing to more efficient practical use.

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Physiological responses of swedish oat varieties to individual and combined drought and heat stress during flowering stage

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Keywords: combined stress, drought, heat, gas exchange, yield

As sessile organisms, plants are increasingly exposed to abiotic stresses, exacerbated by ongoing global climate change. Oat (*Avena sativa* L.), the world's seventh most produced cereal, plays a vital role in Swedish agriculture and food industry due to its high nutritional value. In the field, co-occurrence of drought and heat stress is becoming more frequent that negatively impacting crop growth and productivity. While most research has focused on individual drought or heat stress responses, combined stress scenarios remain underexplored, despite their relevance to real-world conditions. This study investigates the physiological and biometric responses of four widely cultivated Swedish oat varieties (Active, Delfin, Castor, Guld) to drought stress (DS), heat stress (HS), and combined drought-heat stress (DH) during flowering. Drought stress was imposed by withholding irrigation until the soil moisture reached 40% field capacity (FC), monitored via pot weight twice daily. Heat stress (35°C) was applied at anthesis for three consecutive days in a temperature-controlled chamber for 6 hours/day. For DH treatment, drought-stressed plants were simultaneously subjected to heat stress (35°C) for three days while maintaining at 40% FC. A set of control plants (100% FC) was maintained under greenhouse conditions. On the third day of stress, key physiological parameters such as relative water content (RWC), photosystem II efficiency (F_v/F_m), gas exchange (*A*, *E*, *gs*), chlorophyll content (SPAD), biomass, and grain yield were measured post treatment. All stress treatments significantly reduced RWC, F_v/F_m, photosynthetic rate, transpiration rate, stomatal conductance, chlorophyll content, biomass, and grain yield, with the most pronounced effects observed under DH. Heat stress alone increased transpiration, stomatal conductance, leaf temperature, and internal CO_2 concentration, but still resulted in decreased yield. Among the varieties, Active and Delfin were able to recover, while Guld showed poor recovery growth under HD stress. These findings highlight the importance of identifying oat varieties with enhanced resilience to combined stresses, providing valuable insights for breeding programs aimed at improving climate change resilience and sustainable cultivation practices.

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P-185

Riboflavin treatment triggers stress-responsive gene networks for enhanced adaptation in Arabidopsis

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Keywords: Riboflavin, vitamin B2, flavins, transcription factors, gene expression, stress response, plant resilience

Riboflavin is the precursor of the flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) which are

vital coenzymes to a wide array of plant metabolic processes. While the exogenous application of riboflavin has been well-documented to enhance plant stress tolerance, the molecular mechanisms underlying this protective effect remain largely unknown. Here, we present a comprehensive transcriptomic analysis of riboflavin-treated Arabidopsis seedlings, revealing significant changes in gene expression related to stress responses, signaling transduction and secondary metabolism. Riboflavin treatment altered the expression of genes within specific cellular functional categories, supporting the role of riboflavin in regulating plant metabolism and enhancing stress adaptation. The transcriptional changes indicate a shift from growth to stress management, potentially downregulating photosynthesis to preserve energy for immediate stress responses and protect against damage from excess light or oxidative stress. Further, we identified a feedback mechanism where elevated riboflavin levels regulate the expression of genes of its own biosynthetic pathway, controlling both its synthesis and chemical conversion processes. Our study provides novel and valuable insights into the gene expression mechanisms underlying riboflavin-mediated stress tolerance and highlights a potential application of exogenous riboflavin as a strategy for improving crop plasticity and adaptation in the face of environmental challenges.

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Unravelling the molecular network involving microRNA in seed dormancy and leaf senescence in response to temperature

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Keywords: MicroRNA (miRNA), seed dormancy, leaf senescence

Seeds are at the core of agriculture and biodiversity and the efficiency of seed germination and seedling emergence are major factors in crop yield and performance. However, the global climatic changes have adverse effect on gemination physiology which ultimately affects the productivity of agrosystems. Meanwhile, the crucial internal developmental process such as leaf senescence significantly contributes to seed filling and overall plant productivity by ensuring efficient nutrient remobilization, primarily nitrogen (N), from aging leaves to developing plant organs including young leaves, flowers, and seeds. In plants, microRNAs (miRNAs) have been reported to play pivotal role in gene regulation in almost all aspects of plant life and stress responses. However, their involvement in coordinating leaf senescence with seed quality traits remains largely unexplored. The analysis of transcriptome and small RNA-sequencing data libraries from *Arabidopsis thaliana* seeds produced at different maternal temperatures identified ten candidate miRNAs showing differential expression pattern in dry and imbibed seeds. Furthermore, the degradome analysis revealed potential target genes of these miRNAs in both dry and imbibed Arabidopsis seeds. Overexpression (OE lines) and knockdown (STTM lines) mutant lines of the selected miRNAs were generated in Arabidopsis for the functional characterization. Notably, OE lines of two miRNAs (miR-I and miR-II) showed premature leaf senescence, whereas their knockdown (STTM lines) results in delayed senescence. Interestingly, miR-I and miR-II also exhibited a significant difference in N and C content of dry seeds between the OE and STTM lines. The future work includes characterization of molecular functions of miR-I and miR-II in leaf senescence and seed traits and cross-talk with environmental factors. By uncovering novel regulatory mechanisms coordinating senescence and seed maturation/dormancy, this study will provide essential insights into plant productivity and offer potential translational applications for crop improvement.

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Timing matters: Optimizing preharvest harvista™ (1-MCP) application to avoid ripening disorders and maintain postharvest quality in conference pears

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Keywords: Harvista™, Conference pear, postharvest quality, ethylene biosynthesis, gene expression, preharvest

The preharvest application of 1-methylcyclopropene (1-MCP), an ethylene-action inhibitor, has emerged as a valuable strategy for enhancing postharvest fruit quality.

While 1-MCP has traditionally been applied in gaseous form after harvest, the development of Harvista™, a liquid formulation, allows for orchard-level application. However, in pears (*Pyrus communis* cv. Conference), the timing of Harvista application remains a critical challenge. Premature treatments can lead to ineffective fruit drop control and, more significantly, result in the "evergreen effect," where fruit fails to ripen postharvest.

This study aimed to determine the optimal timing for Harvista application by investigating changes in ripening regulation and ethylene-associated gene expression. The results demonstrate that applying Harvista seven days before the optimal harvest date (OHD-7), followed by harvest one day post-treatment, significantly delays ripening while preserving desirable quality traits such as firmness and skin color, even after extended cold storage. This timing also suppresses ethylene biosynthesis and perception at both biochemical (ACC, MACC, ACS, ACO) and molecular (e.g., *ERF17*, *SAM1*) levels. A harvest window of up to four days post-application at OHD-7 remains viable, with only slight reductions in efficacy. These findings offer actionable guidance for growers and contribute to understanding the regulatory mechanisms of ethylene suppression in pears. Future research should explore the varietal differences in response to Harvista, and the integration of this approach with other preharvest and postharvest technologies.

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A multi-scale approach to uncover root senescence dynamics in *Brassica napus* L.

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While leaf senescence has been extensively studied as a key determinant of nutrient remobilization in plants, root senescence remains poorly understood, despite its potential importance in shaping overall nitrogen (N) use efficiency. The root system, particularly in *Brassica napus* L. (oilseed rape), not only absorbs nutrients but may also act as a storage organ for subsequent recycling, especially under nutrient stress, due to the presence of the taproot. Using a combination of morphological, biochemical, and proteomic approaches, we investigated the responses of the taproot in *Brassica napus* L. under two different nitrate conditions during the reproductive stage when senescence occurs.

The taproot transiently accumulates large quantities of starch and proteins and is specifically dedicated to nutrient storage. During the reproductive stage, senescence occurs, characterized by reduced starch and protein reserves in the taproot, and associated with increased activity of cysteine, serine, and aspartic proteases, regardless of the nitrogen availability. Proteomic analysis under low nitrogen conditions identified proteases involved in these activities. More generally, senescence-associated proteins (SAPs) and senescence-decreased proteins (SDPs) were detected, some of which overlap with known leaf senescence markers in *Arabidopsis*, indicating conserved molecular features. Altogether, these results uncovered a distinct root-specific senescence program, characterized by the identification of specific senescence markers in the taproot.

This study provides new insights into root aging and nutrient recycling, with potential applications for improving nitrogen use efficiency in crops.

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Long-term effects of plasma-activated water-based seed pre-treatment on osmotic stress tolerance of pea

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Keywords: plasma-activated fluids, seed pre-treatment, osmotic stress

Drought stress is an increasingly significant challenge in agriculture, necessitating innovative approaches to enhance plant resilience and productivity. Plasma-

activated fluids represent a novel, sustainable technology for seed pre-treatment aimed at improving germination, growth, and stress tolerance. This study investigates the effects of plasma-activated water (PAW) seed pre-treatment on osmotic stress tolerance in pea plants (*Pisum sativum* L. cv. Petit Provencal). The reactive oxygen and nitrogen species (RONS) composition of PAW was modulated by zinc ion (Zn) supplementation. Seeds were treated for one day with distilled water (HP), PAW, PA(W + Zn) or Zn, then grown for 10 days before exposure to 72 hours of osmotic stress induced by 20% (w/v) polyethylene glycol (PEG8000). PEG treatment significantly reduced biomass parameters in shoot and root system among which leaf area was significantly improved by PAW seed pre-treatment. Additionally, PAW pre-treatment mitigated PEG-induced reductions in viability and reduced hydrogen peroxide accumulation. Osmotic stress increased *in planta* nitric oxide levels, independently from seed treatments. To further investigate redox dynamics, the levels of additional RONS were analyzed in root tips. The observed PAW-triggered leaf development under osmotic stress prompted the evaluation of photosynthetic performance (photosynthetic pigments, stomatal conductance and chlorophyll fluorescence). In addition, microelement concentration and sugar content were assessed to monitor associated ionic and metabolic changes.

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Unlocking stress memory in tomato: Effects of repeated drought treatments on tomato stress tolerance

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Keywords: eco-physiology, water stress priming, recovery, transcriptome reprogramming

Drought is one of the major climate-change associated stresses that adversely affect the Mediterranean agriculture, critically altering plant productivity and biodiversity. Improving plant adaptation to drought in crop management systems is therefore an impellent need. Though physiological and transcriptional responses of different tomato cultivars to single drought and rehydration time-courses were studied, drought-primed ecological memory has been poorly investigated.

This work was addressed to establish if the application of repeated water deficit could prime the ability of tomato plants to endure severe drought events. To this aim, tomato plants (cv Moneymaker), grown in pots in a greenhouse facility, were exposed to a first round of water deprivation (WS), followed by recovery. A second WS-priming treatment was applied on the same plants (PRIMED) and on another group previously maintained irrigated (UNPRIMED). Well-watered plants were also included and used as control (CTR). For both experiments, biometric and anatomical traits were measured at the beginning and end of WS imposition to evaluate structural changes at the whole plant level. Physiological parameters, such as xylem pressure, leaf gas exchanges and chlorophyll content, were monitored on all plant groups during the whole duration of both WS and recovery time-courses. Leaf and root samples were also collected at the end of the second WS-priming experiment and processed for metabolic and molecular analyses. Following exposure to the second WS input, PRIMED plants were able to maintain higher transpiration and assimilation rates than the UNPRIMED group. Although PRIMED plants had a smaller total leaf area, such adjustment was compensated by a higher stomatal density compared to both UNPRIMED and CTR plants. No significant changes were observed in the content of soluble carbohydrates and osmolytes in leaves and roots collected from the different plant groups in both WW and WS conditions. Conversely, starch accumulation was opposite in the leaves of PRIMED and UNPRIMED plants based on the water regime. Integration of these data with the analysis of whole transcriptome and DNA methylome changes is ongoing to elucidate the establishment of stress memory signals further.

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Plant approaches to overcome mechanical stresses

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Keywords: cell wall, plant fibers, hydroskeleton, plant biomechanics

The mechanical stresses are faced by a plant through all its life. They originate from the external factors, like wind, rain, damage from animals, etc. However, the major source of mechanical stresses is the growth of plant itself, which increases mechanical loads on the formerly developed plant parts. Mechanical stresses also emerge between plant cells in the course of their division and enlargement and are considered to be the important determinants in plant morphogenesis. To build up the biomechanical system, plants employ two major approaches: the formation of the dispersed «hydrostatic skeleton» based on turgor pressure and primary cell walls, and the development of the accentuated skeleton based on thickened cell walls in specialized tissues, like collenchyma and sclerenchyma fibers. The transition between these skeleton types occurs within the development of each angiosperm plant and can also be considered from the evolutionary perspective. Despite the wide distribution of tissues specialized to fulfill a mechanical function in land plants, the major steps in their emergence and development, same as the functioning of the mechanical system in a plant as a whole are rather poorly understood. We have described fiber-like cells in some bryophytes, exemplifying the first steps in the evolution of mechanical tissues. The next steps of fiber development were characterized by the analysis of living fossil *Psilotum nudum* [2] and of numerous angiosperm taxons. The major features that emerged through fiber evolution and largely helped to establish the plant biomechanical system able to withstand the enormous mechanical loads were hiring of the secondary cell walls based on lignin-carbohydrate complexes, development of the ability for the pronounced intrusive elongation, varying distribution of mechanical tissues within an organ, and the "invention" of fiber-specific tertiary cell walls, whose peculiar design and composition provide contractile properties to tissues and are recruited in many physiological situations [3]. In the newest studies, we have employed complementary approaches, including deep anatomy and immunocytochemistry, transcriptomics, low-field NMR, same as atomic force microscopy and an original device for the inverse three-point bending test, and characterized the major events accompanying the transition from the dispersed hydroskeleton to the accentuated skeleton in stem of flax - the model plant that develops sophisticated system of mechanical tissues that hires all three cell wall types. The talk will present current insights into plant fiber evolution, diversity and mechanisms of in planta performance to provide mechanical stability of a plant organism.

This work was partially supported by the RSF grant # 24-14-00383.

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P-192

Insights on the role of arbuscular mycorrhizal symbiosis and plant genotype in the durum wheat tolerance to environmental stress conditions

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Keywords: arbuscular mycorrhizal fungi, durum wheat, environmental stress

Currently, major threats to durum wheat (*Triticum turgidum* subsp. *durum*) cultivation include climate-induced stresses, such as drought, and phosphorus (P) deficiency. These stresses are frequently combined in the Mediterranean and semi-arid regions, where limited soil moisture reduces P availability. Arbuscular mycorrhizal fungi (AMF) offer a promising biological tool to support plant performance under environmental stress conditions, although effectiveness is influenced by host plant genotype, resources in soil, and stress level. Understanding the genotype-dependent responsiveness

to AMF is therefore crucial for the development of real sustainable agricultural systems. Here, two experiments have been performed to assess the durum wheat AMF-mediated responses i) to water stress and ii) P deficiency. First, a greenhouse experiment was conducted to verify the responses of eight durum wheat genotypes, including wild types and mutant lines provided by UNITUS, inoculated with *Rhizophagus irregularis* and exposed to three water regimes (not-stressed, water deficit and waterlogging). Biometric and physiological traits, including the pot relative water content (RWC_{pot}, %), were monitored and, at the end of stress period, leaf and root tissues were collected for subsequent molecular (RNA-seq) and biochemical analyses. Half of the root system was stained with cotton blue 0.1% and colonization has been evaluated under a microscope. Preliminary results showed that four genotypes (Svevo WT, Kronos WT, Kronos LCYE and Kronos MRP3A$^-$B$^-$) were influenced not only by the stress conditions, but also by the presence of the AMF, showing genotype-dependent responses. A second parallel experiment was dedicated to evaluate three widely cultivated Tunisian landraces (Hadbda, Chili, Mahmoudi) and three commercial cultivars (Salim, Maâli, Marco Aurelio), which were inoculated with *Funneliformis mosseae* and grown in greenhouse under low and high P availability. Biomass and AMF colonization were assessed, and RNA-seq of root tissues revealed gene expression patterns related to P response and AMF symbiosis. Overall, both studies highlight genotype-dependent AMF benefits under stress and contribute to identify resilient lines for low-input agriculture. Ongoing analyses will aim to decipher regulatory networks that drive symbiosis and stress adaptation.

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Exploring the potential role of nitric oxide in regulating nitrate-induced adaptation to waterlogging stress in cucumber

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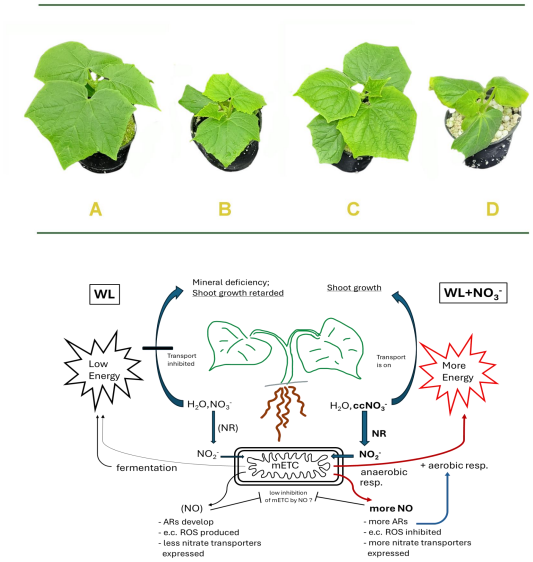
Keywords: waterlogging, flooding, nitrate, nitric oxide, *Cucumis sativus* L.

The waterlogging (WL) stress and its mitigation by nitrate (NO_3^-) were studied in a commercial cucumber hybrid line called 'Joker'. The hypoxic condition was assured at the root zone during the WL stress. WL resulted in anaerobic metabolism in roots as became evident by development of adventitious roots. Higher H_2O_2 and malondialdehyde accumulation were detected in root samples of the waterlogged plants; while shoot growth was inhibited, showing signs of oxidative stress. WL upregulated key hypoxia-responsive genes, including *CsRAP2.3*, *CsRBOHs*, and *CsHem3* phytoglobin. Nitrate supplementation restored shoot growth, reduced oxidative stress, and suppressed fermentative metabolism in roots. Nitrate uptake and transport was improved in a direct relationship with increased NRT1 gene expression. Microscopy and phytochemical analysis showed NO accumulation in the root tissues. Depleting NO with cPTIO confirmed its role in stress mitigation, as AR formation, leaf nitrate content, and protein nitration decreased, and the nitrate transporter gene expression was strongly downregulated. In this study, we could demonstrate that nitrate is able to induce NO-mediated alleviation of WL stress in cucumber and show that the nitrate supplementation facilitated the energy crisis in roots through a combined effect on anaerobic respiration and AR formation. An improved root function and nitrate transports are believed to be likely contributed to shoot growth recovery which was affected by waterlogging. Additionally, we could also show that the NRT1 gene expression under WL was NO-responsive, confirming the role of NO in nitrate-induced stress tolerance.



Cucumber seedlings grown under control (A) and waterlogging stress

(B) after treatment with 20 mM nitrate (C) and 20 mM nitrate plus cPTIO (D).

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The investigation of potential alleviate agents for seed priming in heavy-metal treated *Amaranthus cruentus* L.

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Keywords: amaranth, priming, seedlings, germination

Efficient, fast and uniform seed germination and seedling establishment is the first step for successful growth and production of any crop in agriculture and commercial horticulture. Seed priming seems a promising technique to produce robust plantlets, able to face multiple stresses that crops are exposed to in the field environment [1]. This work aimed to examine and compare four potential priming agents; namely melatonin, selenite, spermidine and salicylic acid. All of them represent powerful antioxidants and should have alleviating effects on plants suffering from heavy metals toxicity [2].

The Slovak cv. Pribina of grain Amaranthus cruentus L. was used to investigate the effect of all potentially beneficial agents. The immersion in water-solutions in several concentrations lasted 24 hours, with 100 µM CdCl2 as a stressor. The germination rate, germination index, seedlings fresh weight and mean radicle length were analyzed after 7 days.

The results showed that lower concentrations of spermidine (≤25 µM) slightly improved fresh weight and germination, but did not affect radicle length. On the other hand, almost all parameters increased after application of melatonin in higher concentrations (≥50 µM). Selenite and salicylic acid showed to be unsuitable as none of the studied parameters was significantly higher compared to stress conditions. The future research is oriented toward studying selected elements supplementations applied simultaneously with the stressor(s) and investigating their role in the modulation of stress tolerance.

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P-195

Functional analysis of green algal serotonin N-acetyl transferases in *Oryza sativa*

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Keywords: serotonin-N-acetyltransferase, green algae, transgenic rice

Serotonin-*N*-acetyltransferase (SNAT), a key enzyme in the biosynthesis of melatonin, is found in all living organisms. It catalyzes the acetylation of serotonin to produce *N*-acetylserotonin (NAS) and the acetylation of 5-methoxytryptamine (5-MT) to form melatonin. Melatonin, known for its potent antioxidant properties, is hypothesized to have evolved under the primordial conditions of early Earth, which were characterized by intense ultraviolet radiation, high oxygen levels, and abundant reactive radicals. Initially, melatonin likely served to protect organisms from environmental stresses and, over evolutionary time, diversified to regulate sleep, circadian rhythms, immune responses, and stress tolerance. Phytomelatonin is believed to have originated

from cyanobacteria, the earliest known photosynthetic organisms. Notably, plant *SNAT* genes show no homology to animal *SNAT* genes, suggesting independent evolutionary origins. Phylogenetic analysis of the Oryza sativa *SNAT1* gene revealed the presence of a homologous *SNAT* gene (*CrSNAT*), as well as an animal SNAT homolog (*CrAANAT*), in the genome of Chlamydomonas reinhardtii, a unicellular green alga considered to represent a common lineage of plant and animal ancestors. Recombinant CrSNAT expressed in Escherichia coli exhibited SNAT enzymatic activity, with a Km of 358 μM and a Vmax of 185 pmol/min/mg of protein when using serotonin as a substrate. Moreover, transgenic rice (Oryza sativa) plants overexpressing *CrSNAT* exhibited increased melatonin production and enhanced tolerance to mannitol-induced osmotic stress compared to wild-type (Dongjin) rice plants. In conclusion, CrSNAT was confirmed to function as an N-acetyltransferase for serotonin and 5-MT, which are precursors of melatonin. These results demonstrate that both plant SNAT and animal AANAT coexist in Chlamydomonas, a green alga that represents a common ancestor of animal and plant cells. This finding offers valuable insights into the evolutionary acquisition of additional melatonin biosynthesis isogenes and the divergence of melatonin biosynthetic pathways.

P-196

Nuclear PP2A B′η drives dephosphorylation of spliceosome subunits to mediate alternative splicing following heat stress

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Keywords: phosphatase, heat stress, alternative splicing

Dephosphorylation of spliceosome components is an essential control step for intron removal from pre-mRNA, thereby controlling gene expression. However, the specific phosphatase responsible for this process has not been identified. Here, we show that *Arabidopsis thaliana* (Arabidopsis) PROTEIN PHOSPHATASE 2A B′η (PP2A B′η), a B subunit of PP2A, associates with the spliceosome B to C complex and recognizes spliceosome components via a conserved binding motif, enabling their dephosphorylation. This dephosphorylation is crucial for proper splicing of retained introns in heat stress-responsive genes, mediated by PRE-MRNA PROCESSING FACTOR 18a (PRP18a), one of the PP2A interactors. Genetic inactivation of PP2A B′η abolished thermotolerance during seed germination and resulted in widespread intron retention in heat stress–responsive genes. Conversely, overexpression of PP2A B′η conferred enhanced thermotolerance, accompanied by efficient removal of the retained introns under heat stress. We demonstrate a central role for a B regulatory subunit of PP2A in dephosphorylating spliceosome components, regulating alternative splicing, and facilitating acclimation to heat stress by targeting specific spliceosome subunits that activate pre-mRNA splicing.

This work was supported by the Korea Research Institute Bioscience and Biotechnology (KRIBB) Research Initiative Program (grant nos. KGM1082511).

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Characterisation of osmotic stress response of *Aegilops biuncialis* compared to *Triticum aestivum* Mv9kr1

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Keywords: Aegilops biuncialis, osmotic stress, polyamines, antioxidants

Drought-induced osmotic stress significantly reduces the growth and worldwide yield of bread wheat (*Triticum aestivum* L.). Its wild relative, goatgrass (*Aegilops biuncialis* Vis.), includes genotypes with enhanced stress tolerance, making them valuable genetic source for wheat improvement. This study investigated the physiological and biochemical responses of two *Aegilops* accessions (*Ae.b.* 382 and *Ae.b.* 642) collected from distinct agroecological habitats, compared to a Martonvásári bread wheat (Mv9kr1) genotype.

Seedlings were subjected to polyethylene glycol (PEG) treatment at the two-leaf stage. Under PEG stress, shoot and root length and fresh weight (FW) were significantly affected in Ae.b. 382 and Mv9kr1, whereas Ae.b. 642 demonstrated greater resilience, particularly in root growth. Enzyme activities, including GR (glutathione reductase), APX (ascorbate peroxidase), MDHAR

reductase), APX (ascorbate peroxidase), MDHAR (monodehydroascorbate reductase), and DHAR (dehydroascorbate reductase), exhibited genotype-specific responses. Ae.b. 642 maintained higher basal activity of APX and DHAR, compared to the other genotypes. Among the studied hormones, the auxin levels increased in Ae.b. 382, while they decreased in Ae.b. 642 and Mv9kr1. However, reduced SA (salicylic acid) levels were found in *Ae.b.*, but their increase or stagnation was observed in Mv9kr1. JA content generally decreased, with the exception of *Ae.b.* 642, while ABA content showed strong increase in all genotypes. Thiol content varied widely across genotypes, with Ae.b. 642 displaying lower concentrations of most thiols in shoot tissues. Unlike of Mv9kr1, the γ-glutamyl-cysteine content significantly increased due to the treatment in roots of both *Ae.b*. genotypes. Phenolic compounds exhibited complex changes under PEG treatment, with distinct patterns observed across genotypes, particularly in vanillin and its derivatives, syringic acid, luteolin and its glucoside conjugates, and in phaseic acid content. Minor alterations were found in the expression of *PIN1 (auxin efflux carrier component 1)*, *NAM (NAC transcription factor NAM-D1)*, *P5CS (Δ -1-pyrroline-5-carboxylate synthase)*, *MYB2 (myb histone 2-like)* genes, while changes in *AUX1 (auxin transporter-like protein 1)*, *ZAT8 (zinc finger protein ZAT8-like)* and *GST (glutathione S-transferase)* expression were more drastic, indicating potential regulatory responses to osmotic stress. Collectively, these results highlight the variability in response to PEG-induced osmotic stress between *Aegilops biuncialis* and wheat, providing insights into the molecular mechanisms of drought tolerance in these genotypes.

This work was funded by the National Research, Development and Innovation Office (TKP2021-NKTA-06, K135057).

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OsFKBP20-1b maintains OsUPF1 and OsUPF2 stability to promote degradation of aberrant transcripts upon dehydration stress

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Keywords: drought stress, OsFKBP20-1b, mRNA stability

Nonsense-mediated mRNA decay (NMD) ensures cellular survival under stress conditions by removing transcripts with premature stop codons. Here, we investigated the role of OsFKBP20-1b, a splicing factor, in the dehydration response in rice. We demonstrate that OsFKBP20-1b interacts with the essential NMD components, UP-FRAMESHIFT 1 (OsUPF1) and OsUPF2, stabilizing them and thus enhancing their capacity to degrade aberrant transcripts under dehydration stress. We confirmed this interaction through bimolecular fluorescence complementation (BiFC) and co-immunoprecipitation assays. Extensive ribosome profiling and transcriptome deep sequencing analyses revealed that OsFKBP20-1b influences not only alternative splicing of transcripts but also translational efficiency of mRNAs encoding stress-responsive genes. Importantly, we show that OsFKBP20-1b substantially enhances the efficiency with which OsUPF1 and OsUPF2 degrade NMD-sensitive transcripts, which is vital for the prompt removal of stress-induced aberrant mRNAs under dehydration conditions. Depletion of OsFKBP20-1b leads to be more susceptible to dehydration stress, underscoring the functional importance of OsFKBP20-1b in stress resilience. Our findings elucidate the integral role of OsFKBP20-1b in mRNA stability and decay during environmental stress. This research provides valuable insights into the molecular basis of drought resilience, offering potential targets for developing drought-tolerant rice varieties.

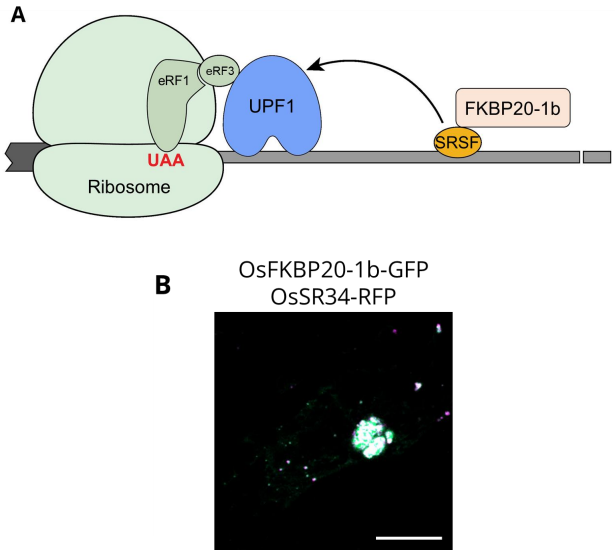

Figure. The emerging role of OsFKBP20-1b in RNA degradation

A. NMD activation model by SRSF and UPF1 in an exon junction complex (EJC)-independent manner. This model was modified from Yi et al., Trends in Genetics (2020).

B. Co-localization of SRSF-like SR34 and its partner FKBP20-1b in processing bodies, where NMD occur.

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P-199

Systemic effects of low-dose ultraviolet treatment on tobacco leaves

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Keywords: UV radiation, systemic effect, ROS signal

The response of an entire plant to a localized external stimulus is referred to as a systemic effect. While the systemic effects of biotic stressors have been well characterized, studies investigating the systemic propagation of abiotic stress responses remain limited. Most existing research on abiotic systemic effects has focused on stressors that cause severe oxidative damage at the site of application. In contrast, our study examines the systemic effects of ultraviolet (UV) radiation (280–400 nm) under conditions that elicit adaptive, eustress responses rather than damage at the site of exposure.

The local effects of UV radiation are strongly influenced by factors such as the applied dose, the spectral distribution of the UV source, and the presence of additional environmental stressors. To investigate acclimative responses to low-dose UV radiation, we used plants grown under relatively low photosynthetically active radiation (PAR) conditions (110–150 µmol photons m^{-2} s^{-1}) in a controlled climate chamber. Four- to five-week-old plants were treated with a broad-spectrum UV source peaking at 311 nm. Under these conditions, UV treatment did not impair photosynthetic performance but led to an increase in UV-absorbing flavonoid pigments on the adaxial leaf surface. Furthermore, the treatment elevated hydrogen peroxide levels and altered antioxidant enzyme activities involved in reactive oxygen species (ROS) regulation (Rácz et al., 2020, Sci. Rep. 10:16303; Czégény & Rácz, 2023, J. Plant Physiol. 280:153884).

Building on these findings, we conducted an experiment in which only a single leaf of tobacco (*Nicotiana tabacum* L. cv. Petit Havana) plants was exposed to UV radiation for two consecutive days (2 h/day; biologically effective daily UV-B dose: 3.4 kJ m^{-2} d^{-1}). We compared responses in the directly irradiated fourth leaf with those in the untreated fifth leaf of the same plant to evaluate systemic signaling.

Our results confirmed the existence of a systemic UV response following low-dose, non-destructive UV treatment. Moreover, they indicate that hydrogen peroxide plays a key role in the signaling processes underlying this systemic response.

This work was supported by National Research, Development, and Innovation Office (grant number NKFIH K-142419).

■

P-200

Variations in root morphology in wheat-pea intercrops under different stress conditions

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Keywords: drought stress, *in situ* root characterization, winter wheat

The study of root system morphology and growth dynamics is essential for plant nutrition, physiology, breeding, and ecological research. Minirhizotron (MR) systems enable non-destructive examination of root system structure, depth distribution, and mortality.

Using the MR technique, we investigated various winter wheat *(Triticum aestivum* L.; 'Mv Nádor', 'Mv Kolompos', YQCCP composite population) and pea (*Pisum sativum* L. 'Aviron') plant communities. The experiment was conducted in six 1 m^3 plastic containers within an ecologically managed research site (HUN-REN ATK, Martonvásár) on chernozem-type soil. MRs were installed horizontally at depths of 20, 50, and 80 cm. Plants were grown under both optimal water supply and drought stress conditions. Root measurements were performed from the early vegetative stage to full maturity using a CI-600 root scanner. Image analysis was conducted with RootSnap! software. Leaf chlorophyll content was assessed with a SPAD-502 meter. We monitored root length (RL) and root surface area (RSA) development and determined the shoot dry mass (SDM) and grain yield (GY) of wheat plants.

Root length (RL) and root surface area (RSA) increased until flowering, followed by a moderate decline. The presence of pea plants reduced the maximum RL and RSA values, with the extent of reduction varying by wheat variety. Drought stress significantly increased root size. Both plant association and drought stress led to a reduction in wheat shoot dry mass (SDM) and grain yield (GY). The presence of peas also resulted in a decrease in the chlorophyll content of wheat flag leaves. Water deficiency further reduced SPAD values, particularly in the variety 'Mv Nádor'. Among the tested varieties, 'Mv Nádor' exhibited the highest tolerance to drought stress

in terms of shoot biomass and yield retention. This drought tolerance was also evident when grown in association with peas.

Our findings indicate that plant association can reduce crop biomass and yield under stress conditions, with the extent of the impact depending on the wheat variety. The MR method provides a valuable tool for the comprehensive assessment of root dynamics and plant responses under different cultivation systems and environmental conditions. It supports breeding programs, variety selection, and the evaluation of plant compatibility in mixed cropping systems.

We conducted our research as part of the ReMIX project within the European Union's Horizon 2020 research and innovation framework program.

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Identification of drought stress responsive regions in the *SINAT2* promoter

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Keywords: abscisic stress, Arabidopsis, guard cell, promoter, SINAT2

Plant growth and productivity are often constrained by various biotic and abiotic stress factors, with drought stress being one of the most critical challenges affecting global agricultural yields. To adapt to these challenging environmental conditions, plants have evolved a variety of resistance mechanisms, with transcriptional regulation playing a crucial role in responding to external stimuli such as drought stress. This regulation is mediated by specific *cis*-acting elements located in promoter regions, which are essential for controlling the expression of stress-responsive genes. In *Arabidopsis thaliana*, the role of the *SEVEN IN ABSENTIA OF ARABIDOPSIS2* (*SIANT2*) promoter in response to drought and osmotic stress has been covered. To further investigate the activity of the *SIANT2* promoter, I conducted β-glucuronidase (GUS) reporter assays. The GUS protein expressed by *SINAT2* promoter was observed in various plant tissues, including leaf veins, and vascular tissues. Notably, GUS staining demonstrated that the *SIANT2* promoter is specifically expressed in guard cells, with its activity significantly increased under osmotic stress conditions, such as abscisic acid (ABA) treatment. This finding suggests that the *SIANT2* promoter responses to drought and osmotic stress signals. This study highlights the significant role of *SIANT2* in regulating transcriptional responses to abiotic stress in plants. Through promoter-mediated transcriptional regulation, *SIANT2* affects guard cell activity, thereby enhancing the overall resilience of plants to environmental stress factors. These findings indicate that *SIANT2* may serve as a potential target for improving plant tolerance to abiotic stresses, such as drought.

P-202

Biochemical markers in the assessment of abiotic stress tolerance in cucurbita plants

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Keywords: abiotic stress, antioxidants, Cucurbita, drought, leaves, pumpkins

The purpose of this study was to compare biochemical stress markers in leaves of *Cucurbita maxima* (14), *C. moschata* (11), and *C. pepo* (11) accessions grown in stressed (drought) and non-stressed conditions (optimal irrigation). The expected intra- and interspecific variability may suggest the potential of these markers as tools for pre-breeding *Cucurbita* plants for improved abiotic stress tolerance. The proposed biochemical markers of stress, analyzed in the leaves of open-field grown plants (at the generative phase of the development), included lipid peroxidation intensity (LP, a proposed marker of oxidative stress and membrane integrity), proline content (Pro, a proposed marker of drought stress), reduced glutathione content (GSH, a proposed marker of abiotic stress tolerance), total phenolic compounds content (TP), and activity of an antioxidant enzyme - superoxide dismutase (SOD). There was a significant variability in foliar contents of Pro (0.5-8.7 mg/g dry weight) and GSH (1.7-5.7 mg/g fresh weight), as well as LP (38.0-155.7 nmol/g f.w.), among the tested species and accessions. *C. maxima* accessions had a lower change in Pro content between control and stressed

plants, contrary to *C. moschata* accessions, which also had higher LP in both control and stressed plants when compared to the other two species. SOD activity was invariable in all investigated plants (0.5-0.7 U/mg f.w.). Almost all accessions accumulated lower content of TP (10.5-22.9 mg/g d.w.) in leaves of stressed plants compared to respective controls (up to 1.8-fold lower). The results suggest that tested biochemical stress markers, except SOD, might be used to investigate abiotic stress tolerance in *Cucurbita* plants. Bearing in mind that the number of publications that report similar experiments in *Cucurbita* species grown in open field conditions are rather scarce, as well as the fact that the improved varieties require multi-year experimental data, the presented findings could provide a preliminary contribution to research focused on mitigating the effects of abiotic stress in these vegetables.

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P-203

Involvement of key genes in barley's response to aluminum stress

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Keywords: aluminum, barley, stress

Aluminum (Al) is the third most abundant element in the Earth's crust, following oxygen (O) and silicon (Si). In neutral or alkaline soils, Al is incorporated into various non-toxic minerals. However, aluminum dissolves under acidic conditions (low pH), forming highly reactive and phytotoxic species known as Al^{3+} ions. Currently, more than half of the world's soils exhibit low pH, and due to industrialization and the excessive use of fertilizers containing ammonia and other acidifying compounds, soil acidification continues to progress, posing a significant agronomic challenge.

Plants have evolved various response strategies to mitigate Al toxicity, including the exclusion of organic acid anions (OAs) into the rhizosphere to chelate Al^{3+} and the sequestration of Al into the vacuole for detoxification. The Al stress response is primarily regulated by the transcription factor STOP1 (*Sensitive To Proton Rhizotoxicity 1*), 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 Al^{3+}:OA complexes into the vacuole.

Our study focuses on the Al stress response in barley (*Hordeum vulgare*), which ranks fourth globally among cereal crops and is primarily cultivated for animal feed. Barley 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. These investigations utilize mutants identified through the TILLING strategy within our *HorTILLUs* population.

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.

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P-204

Enhancing maize yield: The impact of primary metabolites on drought resilience

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Keywords: metabolic pathways, water deficit, *Zea mays* L.

Primary metabolites are essential for plant growth, photosynthesis, and respiration, allowing them to adapt to and recover from a variety of biotic and abiotic stressors. The purpose of this study was to examine changes in sugar, total protein, amino, and fatty acid content in the grains of maize hybrids with varied levels of drought tolerance, with a special emphasis on increasing grain yield under severe water deficit. The experiment was carried out under both irrigated and rainfed conditions. Long-term water deprivation has been shown to impair sugar metabolism, with drought-sensitive genotypes exhibiting a greater decrease in sucrose, glucose, fructose, maltose, and lactose contents. Drought-susceptible and medium drought-tolerant genotypes displayed a slight decline in palmitic, linoleic, and α-linoleic fatty acids, but oleic and stearic fatty acids increased significantly. In response to water shortage, medium- and drought-tolerant genotypes increased their amino acid levels, particularly methionine, lysine, isoleucine, and leucine, while efficiently integrating these

amino acids into protein chains. This finding was supported by a significant positive correlation between total protein and amino acid content ($p < 0.01$). The increased total protein content in drought-tolerant genotypes influenced the carbon-to-nitrogen (C/N) ratio, allowing enhanced nitrogen assimilation, as evidenced by significant negative correlations between sugar and total protein content ($p \leq 0.01$) and between sugars' and amino acids' content ($p \leq 0.01$). Identifying sensitive metabolic pathways associated with seed reserves and grain-filling processes in maize during prolonged water shortages provides useful directions for designing strategies to improve grain yield and seed quality. The capacity to predict crop performance under future harsh climate conditions could greatly benefit the identification of features suitable for breeding efforts aimed at developing sustainable, climate-resilient, and high-yielding maize genotypes.

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P-205

Vitamin B_6-dependent acclimative responses of *Arabidopsis thaliana* developed under different natural UV environments

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Keywords: ultraviolet radiation, vitamin B_6, antioxidant

Ultraviolet radiation (UV, 280–400 nm) accounts for only about 3% of sunlight, yet it plays a crucial regulatory role in numerous plant developmental and stress response pathways. UV can modulate stress responses through both UV-sensitive photoreceptors (such as cryptochrome, phototropin, or UVR8) and reactive oxygen species (ROS). UV exposure enhances the synthesis of flavonoids, hydroxycinnamic acids, certain antioxidant enzymes, or PDX proteins, the latter being responsible for vitamin B_6 biosynthesis. In addition to its fundamental role in hundreds of enzymatic reactions, vitamin B_6 also contributes to plant stress responses as an antioxidant.

In growth chamber experiments, supplementary UV radiation is typically applied using artificial light sources that differ in spectral quality from sunlight, often with UV:PAR ratios that differ from ambient light conditions. Under such circumstances, impaired B_6 biosynthesis led to increased damage of photosystem II (PSII), largely due to an inadequate antioxidant response against hydrogen peroxide [1].

The work presented in this study investigated the vitamin B_6 dependency of acclimation to natural UV environments. Seeds of *A. thaliana* C24 (wild type) and *rsr4-1* (B_6-deficient mutant ecotype) genotypes were sown in autumn 2022 and grown outdoors for six weeks under plastic UV filter filters (a) transmitting full sunlight, b) excluding UV-A, or c) excluding the whole UV spectrum). In *rsr4-1* leaves, the hampered vitamin B_6 synthesis resulted in a UV-independent limitation of PSII quantum efficiency. In order to compensate for this, mutant plants maintained more efficient regulated non-photochemical quenching to avoid oxidative damage. Furthermore, the presence of UV-B altered the distribution of energy dissipation between regulated and non-regulated pathways regardless of genotype.

In contrast to typical plant responses to UV, *rsr4-1* showed elevated superoxide dismutase (SOD) and unaffected phenolic peroxidase (POD) activities, resulting in elevated intracellular hydrogen peroxide levels. Although flavonoid levels were lower in *rsr4-1* leaves due to the B_6 mutation, UV-B exposure still triggered an increase in flavonoids. These results suggest that the deficient POD response in *rsr4-1* under UV-B is not due to limited substrate availability, but rather to a B_6-dependent limitation of POD synthesis or activation.

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P-206

An F-box protein is involved in plant abiotic stress responses by controlling VAMP721/722 levels

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Keywords: abiotic stress, VAMP721/722, VIFBP

VAMP721/722 are the exocytic R-SNAREs that are involved in responses to various external stresses in plants. We previously found that the abiotic stress hormone, abscisic acid (ABA) down-regulates VAMP721/722 levels in Arabidopsis. Little change in *VAMP721/722* transcript levels by ABA but the inhibition of ABA-induced decrease in VAMP721/722 abundance by the 26S proteasome inhibitor MG132 support that ABA post-translationally degrades VAMP721/722 likely via the ubiquitin-proteasome pathway. To understand this, we performed a yeast two-hybrid screening with VAMP722 as a bait and isolated an F-box protein called VAMP722-interacting F-box protein (VIFBP) as a VAMP722-interacting protein. In two independent *vifbp* lines, we found that VAMP721/722 levels are higher than those in WT plants, indicating that VIFBP is indeed involved in regulating VAMP721/722 levels in plants. Interestingly, *vifbp* plants are more resistant to ABA as well as some abiotic stresses than WT plants, likely resulting from the elevated VAMP721/722 levels. The in vitro and in planta interactions between VIFBP and VAMP722 suggest that plants appropriately respond to abiotic stresses at least in part by regulating VAMP721/722 levels via VIFBP. We recently found that ABA also post-translationally controls VAMP721/722 levels in Chinese cabbage. Since the deletion of VIFBP results in better resistance to abiotic stresses in Arabidopsis, we are now trying to remove VIFBP in Chinese cabbage by the CRISPR/Cas9 system. We hope that a VIFBP-deleted Chinese cabbage would become more resistant to abiotic stresses like the Arabidopsis *vifbp* mutants.

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P-207

ACS7 enhances salinity and drought tolerance in *Arabidopsis thaliana*

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Keywords: ethylene, ACS7, abiotic stress, drought, *Arabidopsis*

Ethylene is an essential hormone that regulates plant growth, development, and stress responses. In its biosynthesis process, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) acts as a key enzyme. In this study, we used the wild-type (WT) and various *acs* mutants to reveal the effects of drought and salt stress on the ethylene biosynthetic pathway and understand the mechanism. Through plate screening, *acs7* plays an important role in drought and salt stress responses. Consequently, WT and *acs7* mutants were treated for 14 days under drought and salt stress environments, and gene expression analysis was performed using qRT-PCR. Additionally, ethylene production under salt stress environments was measured using gas chromatography. It was confirmed that *acs7* showed greater sensitivity to drought and salt stress conditions compared to WT, and *DREB2A* expression was also lower in the *acs7* mutant. These results suggest that *ACS7* plays an important role in these stress conditions.

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P-208

The role and mechanism of tomato NAC50 transcription factor in cold stress tolerance

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Keywords: wild tomato, *S. habrochaites*, cultivated tomato, promoter variation, NAC50, MYB3R

Tomato (*Solanum lycopersicum*) is a globally important economic crop originating from the Andean region of South America. Continuous evolution and domestication have led to phenotypic variations among different cultivars. In this study, cultivated tomato (*S. lycopersicum*) and wild tomato (*S. habrochaites*) were used as experimental materials. We identified the *SlNAC50* gene, which exhibited decreased expression under low-temperature stress in cultivated tomato but increased expression in wild tomato. Since the coding regions were highly similar, we compared the promoter sequences and discovered a 70-bp insertion in the upstream region of the cultivated tomato *SlNAC50* promoter, absent in the wild counterpart. Further experiments revealed that a repressive transcription factor, *SlMYB3R*, binds to this 70-bp fragment and negatively regulates *SlNAC50* expression in cultivated tomato. Subsequent functional analyses demonstrated that *SlNAC50* directly binds to the promoters of *SlCBFs* and activates their expression, thereby enhancing cold resistance in tomato.

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P-209

The differential action of the Asada-Halliwell pathway and reactive aldehyde detoxifying enzymes under severe heat and root anoxia

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Keywords: reactive carbonyl forms, heat, flood, wheat

Over the past decade, we have seen more and more how summer heat, drought and sudden heavy rainfall can cause severe stress for crop yields. These factors are harmful on their own, but when they occur together and suddenly, their effect is magnified. These abiotic stressors cause systemic oxidative stress. In addition to ROS, reactive carbonyl forms (RCFs, aldehydes and ketones) are also formed as a result of the chemical reactions of ROS with lipid molecules. Many of the lipid peroxidation products are commonly referred to as reactive electrophilic species (RES) because they contain an electrophilic α, β-unsaturated carbonyl group. To cope with the detrimental effects of RES, plant cells use a variety of non-enzymatic and enzymatic detoxification systems. The latter are less well understood, especially under co-stresses such as the combination of heat and flooding (here: root anoxia).

Our study focuses on the changes in the specific activities of glutathione reductase (GR), glutathione S-transferase (GST), ascorbate peroxidase (APX), catalase (CAT) and aldo-keto reductase (propionaldehyde substrate), alkenal-alkenone reductase (methylvinyl ketone substrate, a heat-inducible RCF) and glyoxalase I and II (methylglyoxal and S-D-lactoylglutathione substrates) under different abiotic stress conditions (heat, root anoxia, their combination) in two Hungarian winter wheat cultivars. Seedlings were grown under normal growth conditions [20°C, PPFD = 250 (µmol m^{-2} s^{-1}], 16/8 L/D period, normal irrigation) for three weeks. The plants were then stressed in three different ways. Either the entire root zone was flooded with water at 20°C, or young plants were exposed to 40°C air temperature for 12 hours and the latter combined with flooding of the root zone. Based on the multivariate analysis of different enzyme activities, we attempted to establish a model for the operation of this enzymatic ROS and RCF scavenging system in two local bread wheat cultivars.

The study was funded by the Hungarian National Research, Development and Innovation Office (Grant Agreement No. K145879).

P-210

Antioxidant capacity and membrane permeability under rewetting recovery of porella platyphylla, a desiccation-tolerant liverwort

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Keywords: antioxidant capacity, desiccation tolerance, *Porella platyphylla*

Relatively constant photosynthetic pigment content (chlorophyll *a*, chlorophyll *b*, and carotenoids) in *Porella platyphylla* (L.) Pfeiff. when experiencing frequent desiccation/ rehydration cycles supports photosynthetic efficiency, directly influencing its growth and survival, and is essential for resilience against oxidative stress and its ability to maintain cellular integrity. When actual pigment composition reports a healthy physiological condition and functionality, the homoiochlorophyllous desiccation tolerance 'machinery' plays a crucial role in the antioxidant defence mechanisms of the liverwort. After a short period of desiccation (1 week), an approximately 200% increase in DPPH inhibition was shown during the first hour of rehydration, indicating a substantial enhancement in antioxidant activity. Folin-Ciocalteu assay revealed exceptionally high polyphenol content for *P. platyphylla*, whose values were significantly higher than those typically reported for a wide range of plants. There is a need for further research to explore the specific types of polyphenols present in *P. platyphylla* and their bioactive properties, or to determine the potentially overestimated polyphenol content. The antioxidant capacity of *P. platyphylla* in mg $FeSO_4$ equivalent appears to be relatively low. However, it shows a remarkable increase in the antioxidant capacity after 48 hours of rehydration, with an increase of almost 900% compared to the initial 24 hours. The difference in the results from the two antioxidant capacity assays (DPPH, FRAP) for the same sample can be attributed to the specific types of antioxidant molecules present in *P. platyphylla*. GC-MS analysis has revealed the presence of several metabolic compounds, whose amounts decreased during rehydration. D-turanose was

identified as a substantial component. Membrane permeability after a short period of desiccation in *P. platyphylla* recovered at 90% during the 48 hours of rewetting, while after a medium-long period of desiccation, the recovery was 50%, and only 5-10% after a very prolonged desiccation.

P-211

Effect of nickel on phosphatase and peroxidase activities in harvesting crops and on the peroxisome organization of *Arabidopsis thaliana*

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Keywords: nickel, phosphatase and peroxidase activities, laser confocal and transmission electron microscopy

Heavy metal contamination of agricultural soils can be natural or man-made origin (industrial sites, using of fertilizer), accumulating in crops grown there. Nickel is essential for plants at low levels, but at higher concentrations it has a toxic effect, leading to chlorosis.

Our experiments investigated the effect of Ni in barley (*H. vulgare*), bean (*P. vulgaris*) and pea (*P. sativum*) plants grown on 21-day-old sand culture in the concentration range of 0-50 ppm. In addition to measuring length and fresh weight (phenetic data), we determined protein (mg/g fresh weight), acidic phosphatase, triose phosphatase, ß-NAD diphosphatase and peroxidase activity. Changes of bean at cellular level were determined by transmission electron microscopy. Ni effect was studied in apple shoots 'Húsvéti rozmaring' in vitro on shoot proliferating MS media containing Ni at the same concentration range. Besides these, the effect of Ni on the number of peroxisomes and peroxule formation was studied in 14-days-old YFP peroxisome fusion protein containing *A. thaliana* model plants.

After nickel treatment acidic phosphatase activity increased in barley and pea. Triose phosphatase activity increased as a response of Ni treatment in barley and pea. When barley and pea were treated with Ni ß-NAD diphosphatase activity increased. Peroxidase activity decreased in all studied plants when the nickel concentration increased. The number of peroxisomes decreased by 53.6 % in *A. thaliana* even on medium containing 3.13 ppm Ni as compared to the control. When YFP-peroxi Arabidopsis was treated with 3.13 Ni, peroxule formation was detected. In parenchymatic cells of the stem of bean cell organelles as nucleus, mitochondria, chloroplasts and peroxisomes were visible, but parenchymatic cells of stem of bean treated with 25 ppm Ni were nearly empty.

Ni had mild effect at low concentration (3.13 ppm), at higher concentration peroxidase activity decreased, while acidic phosphatase, triose phosphatase and ß-NAD diphosphatase increased. In YFP-peroxi Arabidopsis peroxule formation was seen.

P-212

Potential link between the activity of nuclear cap-binding complex and regulation of photosynthesis under drought at booting stage in barley

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Keywords: photosynthesis; barley; cap-binding complex

Under drought conditions, the efficiency of photosynthesis process is impaired, leading to reduced biomass accumulation and diminished yield. Recent studies investigating the role of the cap-binding complex (CBC) across various plant species have demonstrated that mutations in genes encoding CBC components can confer drought resistance. Furthermore, these mutations are associated with increased sensitivity to abscisic acid (ABA) during germination, highlighting the involvement of CBC in ABA-mediated stress responses. In our study, we subjected wild-type (WT) spring barley cv. Sebastian and its TILLING-derived mutants in CBC-encoding genes to drought stress at the booting stage, a crucial developmental phase for yield determination. Our findings revealed that the *hvcbp80.b* mutant, harboring a mutation in the gene encoding the larger CBC subunit, exhibited higher photosynthetic efficiency than WT, as

indicated by an increased PI_{ABS} (Performance Index for Absorption) parameter already at optimal watering conditions. Notably, this improvement was observed also after drought stress. Transcriptomic analysis of *hvcbp80.b* under drought conditions revealed a distinct expression profile associated with photosynthesis-related pathways. Further investigation identified upregulated genes involved in photosynthetic processes in this mutant, enabling us to construct a protein-protein interaction network linking proteins encoded by these genes to the CBP80 subunit. Our analyses suggest that CBP80 may act as a negative regulator of photosynthesis by interacting with specific photosynthesis-related genes, a phenomenon that is particularly evident under water deficit conditions. Despite the observed enhancement in photosynthetic performance, no significant improvement in yield was recorded for the *hvcbp80.b* mutant. Future research will focus on evaluating the effects of exogenous ABA treatment on barley CBC mutants in response to drought, with the aim of determining whether ABA pretreatment can enhance physiological parameters and yield under water-limiting conditions.

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The activity of PM H+-ATPase undergoes phasic changes during adaptation to salt stress

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Keywords: PM H+-ATPase, salt stress, plant adaptation

Soil salinity severely affecting plant growth and crop yields is one of the major threats to global agriculture and food security. The salt stress adaptation process in root cells goes through stages of Na^+ toxicity alleviating associated with growth arrest and subsequent robust growth recovery. The plasma membrane proton pump (PM H^+-ATPase) is the master-protein for the regulation of the majority of transport-dependent processes in plants, therefore, it is critical for the Na^+ cell detoxication mechanism during adaptation to salinity and also is a core component of the cell elongation growth mechanism. Since PM H^+-ATPase is crucial for both Na^+ toxicity alleviation and growth recovery, our research aims to investigate the PM H^+-ATPase activity in root cells at different time points during the 24 hours of salt stress response and to identify mechanisms by which these activity levels are regulated.

The results of our research show that during the first 24 hours of adaptation to moderate salt stress (50 mM NaCl) in root cells of the model plant *Arabidopsis thaliana*, the level of PM H^+-ATPase activity undergoes phasic changes. After the first hour of adaptation to salt stress, PM H^+-ATPase activity is at its highest level. However, between the 3rd and 6th hour of salt treatment the activity decreases and reaches level observed in the control group. Interestingly, PM H^+-ATPase activity increases again between the 6th and 24th hour of salt stress response. Further analyses showed that during the first 24 hours of adaptation to salinity, PM H^+-ATPase in *A. thaliana* root cells is regulated at the gene expression level and its subcellular localization. These results may indicate that PM H^+-ATPase is regulated distinctly at each stage of salinity adaptation. The initial high level of activity during the first hours of salinization can be correlated with the urgent need for Na^+ extrusion from root cells via Na^+/H^+ antiporter. Restoration of ionic and osmotic balance at the cellular level could reduce the urgency of increased PM H^+-ATPase activity. Such conditions also provide a signal for reactivation of previously arrested cell expansion mechanisms, what could explain the subsequent increase in PM H^+-ATPase activity between the 6^{th} and 24^{th} hour of exposure to salt stress.

Our future research will focus on determining the involvement of phytohormones such as brassinosteroids as well as vesicular trafficking as key elements to unravel the precise mechanism of PM H^+-ATPase regulation during adaptation to salinity in root cells.

P-214

Microalgal biostimulants under salt stress: Towards a multi-omics exploration in tomato

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Keywords: salt stress, tomato, microalgae biostimulants

The increasing prevalence of soil salinization represents a significant impairment to global agricultural productivity by constraining plant water uptake and disrupting essential metabolic pathways. In response to this challenge, aqueous extracts derived from microalgae (MAEs), which are naturally enriched with osmoprotectants, antioxidants, and signalling molecules, have emerged as promising biostimulants capable of strengthening plant resilience to abiotic stresses. This investigation was undertaken with the dual aims of discerning the most efficacious MAE from a panel of five microalgal accessions, cultivated under either autotrophic or mixotrophic conditions, for alleviating salinity stress in tomato (*Solanum lycopersicum* L. cv. 'Moneymaker'), and of establishing physiological and molecular mechanisms involved. Initial characterization via FT-IR spectroscopy of the MAEs prepared from the five microalgal strains revealed discernible spectral signatures indicative of differing metabolite profiles. Subsequent 21-day seed germination assays demonstrated the absence of phytotoxic effects across all MAEs; furthermore, extracts derived from autotrophically grown microalgae exhibited a statistically significant increase in germination rates (94%; $p < 0.05$) when compared to their mixotrophically produced counterparts. A subsequent hydroponic screening assay, conducted under both control (1 mM NaCl) and saline (80mM NaCl) conditions, unequivocally identified the extract from *Chlorella* spp. as the top-performing biostimulant. Application of this specific extract notably enhanced the performance of salt-stressed plants, elevating survival rates from 50% to 85% and augmenting shoot biomass by 117.24% ($p < 0.01$). To further validate these findings, a follow-up trial involving tomato plants subjected to weekly foliar sprays of the selected *Chlorella* extract over a five-week period under identical salinity conditions confirmed its efficacy. End-of-treatment measurements revealed a significant mitigation of salt-induced damage, evidenced by improved survival, chlorophyll content, photosynthetic efficiency (F_v/F_m), and biomass accumulation. Collectively, our findings infer that an aqueous extract derived from autotrophically cultivated *Chlorella* spp. substantially alleviates salt-induced stress in tomato, promoting both photosynthetic efficiency and growth under saline conditions. To gain deeper mechanistic insights, we are currently integrating RNA-seq and phytohormonal profiling to delineate the transcript-hormone networks activated by this microalga extract. This comprehensive omics-based approach is anticipated to elucidate the signalling pathways underpinning MAE-mediated salt tolerance and to guide the rational development of optimized microalgae-based biostimulants for sustainable agriculture in saline-affected regions.

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The urea cycle in connection to polyamine metabolism in higher plants: New perspectives on a central pathway

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Keywords: urea cycle, polyamines, putrescine, abiotic stress, stress tolerance, carbamoyl phosphate synthase, reactive oxygen species, ammonium, nitric oxide, signalling, arginine

The ornithine-urea cycle is a biochemical pathway primarily found in animals, where it plays a crucial role in the re-assimilation of ammonium and the removal of

excess nitrogen in the form of urea. In lower photosynthetic eukaryotes, it contributes to metabolic responses during episodes of high nitrogen availability. Unlike animals and lower photosynthetic eukaryotes, in higher plants, the urea cycle is not complete due to the lack of the carbamoyl phosphate synthase-I enzyme that incorporates ammonium into the cycle. Higher plants only possess a type-II carbamoyl phosphate synthase-II that introduces glutamine into the cycle, which is also metabolically linked to arginine and polyamine metabolism.

In higher plants, although historically overlooked, compelling evidence indicates the pivotal role of the urea cycle in different aspects of plant physiology and metabolism, in particular, associated with the metabolism of polyamines, as putrescine, during stress (Urra et al., 2022). Putrescine accumulation is a metabolic hallmark of different types of abiotic stresses, such as drought, salinity, ammonium stress, iron and phosphorus deficiency, and low temperatures. Notably, the exogenous application of polyamines, such as putrescine or spermine, enhances tolerance to abiotic stress, a process in which the free radical nitric oxide appears to play a role.

In contrast to animals, plants do not contain a nitric oxide synthase enzyme, which catalyzes the production of nitric oxide and citrulline from arginine, a reaction which represent a shortcut within the urea cycle. However, arginine may oxidize to N-hydroxyl-L-arginine (NOHA) in a reaction catalyzed by Cyp P450 enzymes, and compelling evidence suggests that NOHA may serve as a substrate to peroxidases to produce nitric oxide (NO). Indeed, peroxidases are known to catalyze the production of NO from other N-compounds as oximes (López-Gómez et al., 2024).

Overall, the urea cycle seems an important pathway in plants, which can accomplish metabolic and signaling functions. In this communication we show the current knowledge on the functionality of the constituent enzymes and metabolites of the urea cycle, and discuss the importance of this pathway in relation to the metabolism of polyamine in higher plants.

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Urra et al. (2022). J. Exp. Bot. 73: 5581–5595.

López-Gómez et al. (2024) Molecular Plant 17, 178–198.

P-216

Hidden hazards of reused wastewater: Physiological and oxidative effects of acetaminophen on rice

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Keywords: pharmaceutical pollutants, acetaminophen, oxidative stress

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 evaluated the impact of a common pharmaceutical pollutant reported in wastewater, acetaminophen, on rice (*Oryza sativa* L.) growth and physiology. For treatment, two concentrations selected were based on reported environmental concentration and the first concentration at which we found this pollutant affected rice 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 compound. These findings emphasize the need for sustainable wastewater management and further research on crop resilience to ensure global food security in the face of increasing environmental contamination.

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Protective sffect of the plant growth regulator MEIA on young einkorn and wheat plants exposed to drowght stress

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Keywords: oxidative stress, drought, PEG, MEIA (β-monomethyl ester of itaconic acid), einkorn, wheat

MEIA (β-monomethyl ester of itaconic acid - a derivative of naturally occurring plant metabolites) is a broad-spectrum plant growth regulator that increases yield and improves the quality of various agricultural crops. The effects of MEIA as a possible protector against adverse stress impacts on young einkorn (*T. monococcum*) and wheat (*T. aestivum*) plants subjected to drought were investigated. The plant growth regulator was applied by spraying, as an aqueous solution, before exposing the plants to stress. Drought was achieved by adding polyethylene glycol (PEG) to the nutrient solution. The samples for analyses were collected after 3 days, when there were visible symptoms of damage. It is well documented that abiotic stress is associated with the formation of reactive oxygen species, leading to oxidative events. To assess the damage caused by PEG treatment and the effect of MEIA, changes in plant biometric parameters (lengths, fresh and dry weight of leaves and roots), stress markers content (such as proline, malondialdehyde (MDA), hydrogen peroxide), electrolyte leakage and the accumulation of some non-enzymatic antioxidants (thiol groups, phenols, anthocyanins) were evaluated. It was found that treatment with MEIA reduces the negative effects caused by applied stress - growth suppression, reduction in plant water content and accumulation of stress markers such as free proline, MDA, hydrogen peroxide, increased electrolyte leakage. The role of the non-enzymatic antioxidants in plant protection was discussed, as well as the sensitivity of the two studied genotypes to the applied stress.

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Genome-wide systematic analysis of WUSCHEL-related homeobox gene family and integrative analysis to excavate their potential roles in developmental and abiotic stress responses

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Keywords: *In silico* analysis, *Picrorhiza kurrooa*, WOX

The *WUSCHEL-related homeobox* (*WOX*) gene family is a group of transcription factors unique to plants, and are distinguished by the presence of a homeodomain which plays crucial role in plant development, *de novo* organogenesis and stress response. *Picrorhiza kurrooa*, an endangered herb native to the Himalayas, holds great ecological and medicinal potential. However, the molecular mechanisms driving its organ development and resilience to environmental stresses are still being explored. Current study aimed to unravel the structural basis, genetic diversification and novel insights into development and stress resilience pathways of *P. kurrooa*. Transcriptome profiling during different stages of *in vitro* organogenesis identified stage-specific gene expression patterns. Further, differential gene expression analysis revealed role of *WOX* gene family in various biological processes. Comprehensive genome wide identification of *WOX* gene family, followed by domain analysis and subcellular localization predictions, showed five WOX and one WUS members distributed between 2 and 7 chromosomes. Moreover, we successfully developed a reliable and efficient method for isolating protoplasts, along with a PEG-mediated transient protoplast transformation system in *P. kurrooa*. Transient overexpression of *PkWOX11* in *P. kurrooa* protoplast and subcellular localization results demonstrated its localization in the nucleus. Overall, this stable and versatile protoplast-based transient expression system, will improve our knowledge on functional characterization of *P. kurrooa* proteins. This advancement accelerates research on potential targets for future biotechnological advancement with particular emphasis on their roles in stress tolerance and secondary metabolite biosynthesis and streamlines molecular breeding processes with high-throughput efficiency for sustainable agriculture and crop improvement.

P-219

Non-autonomous Long Terminal Repeats retrotransposons (LTR-RTs) targeting abiotic stress adaptation in plants

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Keywords: LTR, retrovirales, bioinformatics, heat stress, ABA, RNAseq, sRNAome

Background: LTR-retrotransposons (LTR-RTs) are a large group of transposable elements (TEs) that constitute an intrinsic and significant part of the genomes of many eukaryotic species, especially plants. They primarily belong to the *Pseudoviridae* and *Metaviridae* viral families. The LTR-RTs are first transcribed by RNAPII and may replicate through reverse transcription (RT), followed by integration of the resulting cDNA into a new locus in the genome (copy-paste mechanism). This replication mechanism resembles that of retroviruses, with the key difference being that LTR-RTs do not produce infectious particles capable of moving between cells. Consequently, the original LTR-RT remains in its initial position in the genome, while the new copy integrates elsewhere; this process contributes to host genome expansion and increase of LTR-RT copy number (1).

Non-autonomous elements do not encode the proteins necessary for transposition, however, they can be expressed (but not autonomously pasted) upon internal and external environmental cues. Non-autonomous LTR-RTs are present in many eukaryotic genomes and have been extensively studied in several plant species using computational methods, and specialized platforms now exist to support research in this area (2). Despite these, LTR-RT's regulation and biological relevance at organism and at evolutionary scale is still poorly understood.

Key Findings: To better understand (i) LTR-RT regulation in plant species and (ii) their potential biological impact on host organism, we selected those 69 non-autonomous LTR-RTs located inside or in vicinity of euchromatic gene loci of the *A. thaliana* genome for analysis. To get a hint on their activity, we bioinformatically examined publicly available sRNAome and RNA transcriptome data, as molecular markers associated with these loci. The analysis was performed on datasets obtained under ambient conditions (control, non-treated), or on data obtained under stress conditions (particularly under abiotic stress -mimicking abscisic acid (ABA) treatment or heat stress exposure). Among the identified LTR-RTs, some appeared highly responsive, while others were irresponsive to the applied treatments, implying that at least some of these may be functionally important. Preliminary findings of this investigation have also been extended to selected crop species, such as *Cicer arietinum* (chickpea) and *Hordeum vulgare* (barley).

Future Research Directions: These results suggest the possible involvement of LTR-RTs in epigenetic and/or transcriptional regulation of proximally located gene/loci; this, through an as-yet unidentified epigenetic/transcriptional mechanism may support plant adaptation to environmental stress. Future research will aim to elucidate this mechanism and explore its potential role in improving crop resilience.

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P-220

Investigating plant drought stress response through the integration of public transcriptomic data

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Keywords: RNA-seq, water deficit, cross-species analyses, gene expression, drought-stress

Environmental changes over recent decades have led to numerous challenges for crop yield worldwide,

necessitating adaptation to a range of new conditions. Among these, water deficiency is one of the most critical, significantly affecting plant growth, particularly in hot regions. Therefore, it is crucial to gain a deeper understanding of gene expression patterns and regulatory mechanisms used by plants to cope with this stress. To address this need, we leveraged publicly available RNA-seq data from drought stress experiments conducted on three species of particular interest: two dicots, *Arabidopsis thaliana* and *Solanum lycopersicum*, and one monocot, *Triticum aestivum*, to perform comparative transcriptomics. We focused mainly on leaf and root organs, including only experiments associated with published studies. The collected transcriptomic data, for each species, was utilised for a principal component analysis that revealed no clear separation based on treatment (i.e. control and stress conditions), but rather on BioProject and stress-application method, indicating the presence of batch-effects. However, the employment of established strategies removing the batch-effect reduced this trend, uncovering hidden patterns associated to the drought stress treatment. Following differential expression and enrichment analyses on the perturbed gene sets identified, we highlighted biological processes particularly impacted by the stress, such as "response to water deprivation", "photosynthesis", and "translation". Finally, an orthology analysis led to the identification of shared differentially expressed orthogroups across the three species analysed. The comparative transcriptomics approaches described here contribute to deepen the knowledge about genes and their related pathways involved in the response to drought stress, shedding also light on the genetic regulation implemented by the three examined species. In conclusion, this approach will expand our knowledge on plant responses to drought stress, thereby paving the way for novel breeding applications.

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P-221

Small Paraquat Resistance (SPQ) protein: A multifunctional regulator of abiotic stress tolerance in plants

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Abiotic stresses such as drought, salinity, and oxidative damage pose significant challenges to plant growth and crop productivity. The Small Paraquat Resistance (SPQ) protein has been identified in the salt- and drought-tolerant plant *Lepidium crassifolium*, and was found to be implicated in responses to such stresses. SPQ overexpression in *Arabidopsis thaliana* enhances paraquat tolerance and confers drought resilience. SPQ overexpression decreased the ABA-induced sensitivity of primary root elongation to abscisic acid (ABA) suggesting its role in ABA signaling. Functional disruption of Arabidopsis SPQ via T-DNA insertion and/or CRISPR-Cas9 mutagenesis results in heightened sensitivity to paclobutrazol during germination. Transcript levels of *MAF5* and *FLC* genes were enhanced in the spq mutants, suggesting that SPQ may modulate flowering time regulation.

Subcellular localization of GFP-tagged SPQ showed that the fusion protein is associated to the endomembrane system, including the vacuolar membrane as well as to chloroplasts, suggesting its involvement in diverse cellular processes. Identification of SPQ-associated proteins through immune-precipitation of HA-tagged SPQ and subsequent mass spectrometry suggested that SPQ may interact with various membrane-bound proteins in chloroplasts and the endomembrane system.

Our results suggest, that SPQ is implicated in regulation of various stress- response pathways, through a still unknown way. These insights however suggest that SPQ can be a promising candidate for engineering abiotic stress resilience in crops.

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P-222

Systemic leaf responses to UV via hydrogen peroxide and pathogenesis-related protein1

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Acclimative UV-responses are regulated by multiple pathways, such as photoreceptors or ROS. UV-B is a potent inducer of the plant pathogenesis-related protein PR-1. We already know that UV induces the expression of PR1 [1]. UV generated ROS upregulate the expression level of PR1 but not in the absence of salicylic acid (SA). There is no consensus on the role of SA in this process because the mode of action of ROS could be upstream of SA. SA is one of the main promoters of pathogen related SAR, and H_2O_2 is known as a general signal molecule. In this work we studied the potential role of the above in systemic responses to UV in partially UV-B exposed Col-0 and PR1::GUS plants. 8-week-old plants were half covered by UV-excluding filter. The filter effect was investigated by covered control plants which shown no expression of PR1 according to GUS-staining nor PCR product. Plants were exposed to supplemental UV irradiation (spectrum was centered at 311 nm, ~7 kJ m–2 d–1 b.e.d.) during 3 hours for four days. GUS-staining showed an increase of PR1 in the filtered leaves after 3rd day of UV-treatment indicating its systemic effect. This result was later confirmed by PCR. The H_2O_2 content increased significantly in the covered leaves for the 4th day of UV-treatment. Our results prove that the measured parameters are part of the plant systemic responses to UV. In our next experiment we will use transgenic NahG plants.

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Exploring essential and contaminant distribution in plants: X-ray fluorescence imaging

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Keywords: cadmium, iron, manganese, titanium

Concentrations of essential heavy metals, like all environmental factors, have to be kept within an optimal range to avoid plant stress. Low concentrations may induce deficiency, whereas toxicity arise at high concentrations. Non-essential metals might be toxic as well. The control over their distribution in plant tissues is vital to avoid both deficiency of essential elements, and accumulation induced toxicity. Thus, understanding lateral distribution, compartmentalization, that support the understanding of the underlying transport processes has a prime importance. X-ray fluorescence (XRF) imaging is a powerful non-destructive technique that provides high-resolution elemental maps of flat surfaces such as leaves, enabling the visualization of Fe distribution at organ, and tissues levels. In the current study we applied a Horiba XGT-7200 desktop imaging system for the study of organ-level distributions of essential macro- and microelements. The system enables to reach resolutions down to 2 µm pixel width, depending on the resolution and area settings (Gracheva *et al*. 2022). However, signal intensity obtained from the K emission of essential and trace elements generally does not support the imaging of their distribution. Indeed, obtained X-ray fluorescence still bear valuable information. Accurate data handling is critical for precise interpretation, and strategies for minimizing artifacts, optimizing signal-to-noise ratios, and employing advanced image analysis methods must be used to generate qualitative and semi-quantitative distribution profiles. XRF-based techniques are versatile and can be used to study diverse plant species, providing a comprehensive understanding of Fe metabolism throughout the plant kingdom. We have tested multiple plant models, including *Arabidopsis thaliana*, *Triticum aestivum*, and *Ginkgo biloba* to how essential and non-essential metals can be detected. Using elemental profiling by comparing the natural distribution of essential and trace elements, we can identify potential

interactions and co-localization patterns for a deeper understanding of plant physiology. We highlight the key role of XRF imaging and careful data handling in advancing knowledge of essential and trace metals in plants. This knowledge could be used to address of tolerance to environmental stresses.

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P-224

Physiological and molecular responses of textile hemp (*Cannabis sativa* Santhica 27) to cadmium and zinc, with a particular focus on the analysis of silicon-induced protection mechanism

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Keywords: heavy metals, silicon, hemp, physiology, phytoremediation

Soil contamination by heavy metals, particularly cadmium and zinc, represents a major environmental and health concern, exacerbated by industrial, mining, and agricultural activities. Phytoremediation, an ecological and cost-effective strategy, leverages plants to extract or stabilize these pollutants. Textile hemp (*Cannabis sativa* L. var. Santhica 27) is a promising candidate for this approach due to its deep root system, high biomass production, and non-food applications. Although silicon is not an essential element for plant growth, it has been shown to enhance tolerance to abiotic stresses.

This study investigates the effect of silicon on the response of textile hemp exposed to cadmium and zinc contamination in a highly bioavailable hydroponic system. Silicon was supplied in hydroponic solution as 2 mM metasilicic acid, ensuring full phytoavailability. One week later, cadmium (20 µM) and zinc (100 µM) were introduced. A multidisciplinary approach was used, integrating molecular (proteomic), biochemical, and physiological analyses – including pigment composition, hormonal profiling, and gas exchange measurements – to better understand the adaptation and accumulation mechanisms of hemp in a phytoremediation context.

Contrary to numerous studies highlighting the beneficial role of silicon in mitigating abiotic stress, our results indicate no protective effect and even potential negative impacts on certain physiological mechanisms. Specifically, silicon application led to a reduction in chlorophyll and certain carotenoid concentrations, with no significant improvements in physiological parameters. These findings suggest that silicon does not enhance the tolerance of textile hemp to cadmium and zinc stress, potentially due to the plant's relatively low silicon accumulation capacity.

P-225

Effect of potentially toxic metals on industrial and food crops: A NMR-based metabolomics study

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Keywords: cadmium, germination and seedling development, lead

Potentially toxic metals pose significant risks to seed germination and seedling development, ultimately impacting crop production. When seeds are exposed to these toxic metals, their germination rates may decrease, and seedling growth can be stunted, leading to poor root and shoot development. Understanding the effects of potentially toxic metals on plant growth is vital for developing strategies to mitigate their impact, improving agricultural sustainability and food security. Metabolomics has emerged as an important ally in understanding the biochemical and molecular mechanism of industrial and food crops in response to abiotic stresses, which is crucial for developing stress-resistant plant varieties through targeted breeding and genetic engineering. Herein, this study assessed the physiological, biochemical and metabolomics responses of *Triticum spp.*, *Ricinus communis*, and *Glycine max* to different levels of cadmium (1.0 mM, 2.0 mM and 3.0 mM) and lead (1.5 mM, 3.0 mM and 4.5 mM) during seed germination and seedling development. Exposure to Cd and Pb had a negative impact on seedling growth and

development for all three species. Root and shoot lengths, as well as dry weight were reduced with the increasing concentration of Cd and Pb. However, dry weight was reduced to a lesser extent leading to higher dry weight-to-length ratio with the increasing concentration of Cd and Pb. Variation in chlorophyll content and SOD activity has demonstrated that these species have somehow good plasticity to cope with the stress posed by Cd and Pb. A quantitative NMR-based analytical method for probing polar metabolomics was developed and included the assessment of the effect of lipoprotein removal by chemical delipidation and by chemical precipitation, using two different pulse sequences, and four different number of scans during acquisition. ^{1}H NMR spectra for the calibration curves were acquired for eight points with concentrations ranging from 0.00 to 10000 µM. The method's robustness was assessed by its linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ). Over 30 metabolites were identified and quantified in *Triticum spp.*, *Ricinus communis*, and *Glycine max* different tissues, which included several amino acids, organic acids, stress-related metabolites, and carbohydrates. Multivariate statistical analysis was applied to pinpoint possible biomarkers for plant resistence to Cd and Pb toxicity. The PLS-DA analysis showed that the validated method could differentiate *G. max*, *R. communis*, and *T. spp* tissue metabolomes subjected to normal and stress conditions. Ultimately, the validated method might help to develop cultivars with enhanced resilience and improve agricultural sustainability in changing climates.

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The role of root functional traits in determining tree health under a rain exclusion experiment

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Keywords: drought, root plasticity, soil microbial communities

Tree root system is a key compartment for maintaining forest function and ecosystem services they provide. Water shortage leads to changes in soil physicochemical properties, biotic interactions, and the availability and distribution patterns of carbohydrates allocation, inducing changes in the architecture, morphology and physiology of the roots to optimise resource acquisition. However, these belowground adjustments may come at a cost to aboveground functions´ maintenance, potentially leading to changes in tree health status. Identifying key root functional traits related to water shortage-induced health transitions in trees is essential to reduce tree mortality and, therefore, minimise forest decline. In this work, we aimed to study how rain exclusion-induced changes in soil moisture conditions and microbial communities affect root traits and, consequently, drive shifts in resource acquisition strategies and non-structural carbohydrate (NSC) allocation. We focused on a Mediterranean native tree species (*Quercus faginea* Lam.) and a planted species (*Pinus pinea* L.). To simulate drought scenarios at the tree level, rain exclusion umbrellas (REUs) were installed near Vitoria-Gasteiz (Basque Country, Spain) from March to October 2023. For each species, four trees were subjected to drought simulation, while four served as controls. At the end of the growing season, soil physicochemical properties, soil

microbial diversity, NSC, and root architecture were measured to analyse belowground responses. Results showed contrasting drought response strategies between *Quercus faginea* and *Pinus pinea*, with species-specific adjustments in root traits and differences in microbial community structure. This work highlights the importance of root functional traits and plant-soil interaction mechanisms in mediating species-specific drought responses, offering valuable insights for anticipating tree health transitions under drought scenarios.

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Impact of cold storage on quality parameters, bioactive compounds, and gene expression in different cultivars of berries

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Keywords: soft fruit, quality, low temperature, firmness

The fresh consumption of berries such as blueberries, raspberries, and blackberries has significantly increased in recent years due to their recognized health-promoting properties. Consequently, preserving the quality of these fruits during cold storage has become essential. In this study, we evaluated the effect of low-temperature storage on key quality attributes of two different cultivars of blueberries, raspberries, and blackberries. Parameters analyzed included soluble solids content, titratable acidity, incidence of decay, and mechanical properties related to firmness. Additionally, the levels of health-beneficial compounds such as anthocyanins and phenolics were assessed.Markers of oxidative stress were also examined to better understand the physiological response of the fruits to cold stress. Furthermore, gene expression analyses were conducted to study the regulation of genes involved in cell wall integrity and flavonoid biosynthesis.

The results showed that the response to cold storage was cultivar-dependent. A loss of firmness was observed, accompanied by changes in the expression of genes associated with cell wall integrity. Cold storage also altered anthocyanin and phenolic contents, along with the expression of genes involved in their biosynthetic pathways.

These findings contribute to a better understanding of the molecular mechanisms underlying the response of berries to cold storage and may support the development of postharvest coadjuvant treatments to enhance fruit quality preservation

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Functional role of the E3 SUMO ligase SIZ1 splicing variant in heat stress response: From localization to function

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Keywords: alternative splicing, CNGC6, E3 SUMO ligase, heat stress, SIZ1, SSV2

SIZ1 is a member of the Siz/PIAS-type RING family of E3 SUMO ligases, which play key roles in growth, development, and stress responses in both plant and animal systems. However, splicing variants of SIZ1 have not yet been characterized. In this study, we identified five splicing variants of Arabidopsis SIZ1, encoding three distinct protein isoforms. The SIZ1 gene encodes an 873-amino acid (aa) protein. Among the five SIZ1 splicing variants (SSVs), SSV1 and SSV4 encode identical 885-aa proteins, while SSV2 and SSV5 encode identical 832-aa proteins. SSV3 encodes an 884-aa protein. Subcellular localization analysis revealed that SSV2/SSV5 predominantly localized to the cytoplasmic membrane, whereas SIZ1, SSV1/SSV4, and SSV3 were nuclear-localized. Interestingly, SIZ1 and all SSVs exhibited similar E3 SUMO ligase activities and showed a preference for SUMO1 and SUMO2 as substrates. The transcript levels of SSV2 and SSV5 significantly increased

upon heat treatment, while those of SSV1, SSV3, and SSV4 remained unchanged under various abiotic stresses. Furthermore, SSV2 directly interacted with and stabilized the heat-activated plasma membrane Ca^{2+}-permeable channel protein CNGC6, a positive regulator of thermotolerance, through its E3 SUMO ligase activity, suggesting its role in heat stress tolerance. Notably, transgenic siz1-2 mutants expressing SSV2 exhibited greater heat stress tolerance than wild-type plants, whereas those expressing SIZ1 were more sensitive to heat stress. Given that Siz/PIAS-type E3 SUMO ligases are conserved across eukaryotes, we propose that alternative splicing serves as a key regulatory mechanism modulating their function during development and in response to environmental stresses, including heat stress.

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Testing the potential of protein hydrolysates derived from waste materials as seed primers

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Keywords: seed priming, coriander, waste protein hydrolysates

In recent years, the growing emphasis on sustainable agriculture and circular economy principles has led to increased interest in the reuse of industrial by-products. Waste protein hydrolysates, rich in amino acids, peptides and bioactive compounds, show promising potential as natural biostimulants. Their use in seed priming - a pre-sowing treatment designed to improve seed quality and support early plant development - can provide an innovative and environmentally friendly approach to improving the resilience of established crops. By promoting faster and more uniform germination, increasing stress tolerance and promoting seedling vigour, protein hydrolysates can be valuable tools in agriculture and horticulture. Seed priming with water or, under strictly controlled experimental conditions, especially with aqueous solutions of active ingredients, results in increased germination over a wider temperature range, improved speed and uniformity of seedling emergence and the ability to extend the growing season, which is particularly important under suboptimal conditions.

Coriander (*Coriandrum sativum* L.) is one of the most important spices (aromatic) and medicinal plants, grown commercially in many parts of the world, including Poland. It is an annual herbaceous plant of the celery family (Apiaceae), native to the Middle East and Mediterranean region. In addition to its culinary and therapeutic uses, coriander is a valuable melliferous plant that, when also sown supplementally between monocultures, contributes to improving the biodiversity of agricultural ecosystems. Due to its versatility, coriander is cultivated not only for its aroma and taste, but also for its positive environmental impact and contribution to sustainable agriculture.

The aim of the present study was to test whether protein hydrolysate (industrial waste) applied into the seeds could promote coriander germination under high temperature stress conditions. The waste protein hydrolysates used for seed conditioning were fish collagen (KOL) and sheep wool keratin (KER). Untreated seeds (control) and seeds conditioned with: water (H), collagen solution (KOL) and keratin solution (KER) were used for testing. All seeds were pre-incubated for 9 days at 35°C and then placed at the optimum temperature for germination. The results indicated a higher percentage of germination of treated seeds after stress elimination. The best effect was obtained with 0.5% KOL and 0.5% KER application. The indicated hydrolysates contributed to efficient plant regeneration after high temperature stress. Further testing is necessary, but our results presented here indicate that the methodology used has the potential to protect plants from climate change associated with increased temperature and drought.

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Exploiting barley germplasm of different geographical origin to dissect the mechanisms of their response to drought applied at seedling and heading stages

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Keywords: barley, drought stress, yield, germplasm

Yield of crops under drought stress is significantly reduced, what is evident in modern varieties of cultivated species. This fact is closely related to modern breeding, in

which maximizing yield parameters of new varieties has led to a significant narrowing of genetic variability in other genes, including those predisposing the plant to better cope with water deficiency. Presented study investigates 10 barley genotypes from different geographical origins - including Morocco, Jordan, USA, Italy, UK, Denmark, Norway and Finland - with an aim to broaden the genetic diversity for breeding programs directed toward development of drought-tolerant cultivars.

In this analysis, plants were subjected to water deficit at seedling and heading stages in two separate experiments, with the latter followed by post-harvest analysis. Physiological parameters including Relative Water Content (RWC), chlorophyll, anthocyanin and flavonol content, chlorophyll fluorescence parameters, stomatal conductance and gas exchange - were measured after 10 days of drought and 7 days after rewatering. Our results revealed significant differences among genotypes and highlighting distinct drought response mechanisms. Notably, the Moroccan landrace exhibited a drought escape strategy, characterized by rapid development and shorter spikes. The Jordanian landrace was the most drought-sensitive at the seedling stage showing more than 90% reduction of photosynthesis efficiency. The Danish modern cultivar, despite being highly affected by drought at heading stage, maintained a yield comparable to other genotypes. In contrast, the American old cultivar demonstrated strong physiological resistance at the heading stage but poor recovery, resulting in significantly reduced yield. The Danish landrace maintained moderate photosynthesis during drought despite losing over 30% of its chlorophyll content, emerging as the best-performing genotype at seedling stage. Meanwhile, the British and Italian modern cultivars exhibited above-average recovery at seedling and heading stages, respectively.

The results obtained in these experiments indicate different defense mechanisms or response dynamics of barley genotypes under drought stress. Based on these findings, four genotypes will be selected for an in-depth time-course analyses to characterize their response throughout drought progression. Additionally, transcriptome analysis will be conducted to identify candidate genes potentially involved in shaping drought stress tolerance in barley.

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Rice calcium-dependent protein kinase 17 directly targets sucrose-phosphate synthase 4 to modulate rice cold acclimation

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Plant CDPKs perceive developmental and environmental cues, transducing them via phosphorylation of downstream targets. In a previous study, we showed that OsCPK17 modulates rice cold stress by phosphorylating C/N-related enzymes, such as OsSPS4, OsSPS5, OsNR1, OsFd-GOGAT, OsPDHE1-A, and aquaporins OsPIP2;1/2;6. [1] However, the impact of OsCPK17 on C metabolism remains unclear. This study investigates the functional relevance of OsCPK17-mediated phosphorylation of OsSPS4. Using subcellular localization and bimolecular fluorescence complementation assays, we confirmed that OsCPK17 co- localizes and interacts with OsSPS4, but not OsSPS5 or OsNR1. OsCPK17 localizes to the trans-Golgi network and plasma membrane, while OsSPS4 dynamically localizes in the tonoplast, cytosol, and amyloplast, consistent with its role in sucrose remobilization. The OsCPK17-mediated phosphorylation is not required for OsSPS4's amyloplast localization. We developed a CRISPR/Cas9 KO line for *OsSPS4* which revealed its critical role in cold acclimation, being determinant for proper cold tolerance and sugar levels. The absence of OsSPS4 also impaired plant growth and development in control conditions. Under greenhouse conditions, *ossps4* plants showed delayed maximum photosynthetic efficiency at midday. These findings highlight OsSPS4 role in rice cold acclimation, sugar metabolism, and photosynthetic performance. Additionally, OsSPS4's localization in amyloplasts, where starch is stored and degraded, suggests a role in carbon remobilization during cold stress.

[1] Almadanim, C. et al. (Plant Cell Environ, 2017), doi: 10.1111/pce.12916

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Coordination of shoot and root hydraulics in maize plants in response to elevated atmospheric CO_2 concentration

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Keywords: hydraulic system of plants, spin-echo NMR, cell-to-cell pathway, apoplastic pathway, aquaporins

Coordination of plant hydraulic system components is an important function of plant adaptation to abiotic factors. It is the coordinated and timely response of the plant's hydraulic system that allows it to survive changes in environmental conditions most effectively. This coordination can take place at the level of both long-distance and short-distance water transport. Investigation of the interaction and redistribution of contributions of water transport pathways, identification of key regulators and signalling factors of water transport processes and determining the dynamics of these processes is important for understanding the functioning of the plant hydraulic system under different conditions, including changes in CO_2 concentration. In this context, we investigated the dynamics of transpiration, apoplastic and cell-to-cell water transport in roots of whole maize plants, the functional activity of leaf and root aquaporins, and changes in xylem sap pH, as a signalling factor, in response to a sharp increase in atmospheric CO_2 concentration as a triggering factor. When the atmospheric CO_2 concentration was increased from 400 to 800 ppm, there was a rapid (within 5-7 minutes) decrease in transpiration rate by 40%. At the same time, responses were registered in the roots of the plants. In particular, there were changes in radial water transport in the roots. According to the results of NMR measurements of diffusional decays of water magnetization in the roots, it was shown that after a sharp increase in CO_2 concentration, after about 60-70 minutes, there was a decrease in cell-to-cell water transport in the roots by 18-20%. The intensity of paramagnetic ion penetration into the root apoplast during simultaneous plants exposure to elevated CO_2 concentration was 1.5-1.8 times lower than in control plants, also indicating a decrease in water transport via the apoplastic pathway. It was shown that the expression of some aquaporin genes were reduced under elevated CO_2. The decrease in aquaporin gene expression levels was observed 2 h after CO_2 increasing and occurred simultaneously in both leaves and roots. The PIP and TIP aquaporins that were most responsive to elevated atmospheric CO_2 were identified. Three days after elevated CO_2, aquaporin gene expression levels remained below control levels. Interestingly, the decrease in radial water transport in roots, transpiration rate in leaves, and aquaporin gene expression in roots and leaves was accompanied by changes in xylem sap pH. The obtained results can improve the understanding of the coordination mechanisms of the components of plant hydraulic system in response to elevated CO_2.

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Functionality and structural performance of *Chlorophytum comosum* L. (spider plant) leaves in formaldehyde polluted air

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Keywords: formaldehyde, Chlorophytum comosum, photosynesis

Formaldehyde has been identified as a major indoor air pollutant, with potential carcinogenic and teratogenic properties. It has been documented that levels of formaldehyde in indoor air frequently exceed the maximum permissible limit of 0.1 mg m^{-3}, as stipulated by the World Health Organization (WHO).

In recent years, considerable attention has been directed towards the phytoremediation of HCHO, with a focus on identifying plant species capable of purifying the atmosphere and markedly diminishing the concentration of this compound in indoor environments. It has been documented that a multitude of potted plants, including *Chlorophytum comosum* L. (Spider Plant), have the capacity to efficiently eliminate HCHO from indoor environments.

The present study investigates the structural changes and parameters related to photosynthetic activity in *C. comosum* leaves that have been exposed to exogenous HCHO for 48 hours at a concentration of 20 mg m^{-3}. Scanning electron microscopy observations of *C. comosum* plants revealed no alterations in the morphology of the leaf abaxial and adaxial surfaces. However, a discernible variation in the appearance of the stomata was observed. In the case of plants experiencing stress, the stomata are observed to be reduced in size and exhibit a more square-like shape. However, under conditions of optimal control, the stomata demonstrate an elongated morphology. The application of HCHO caused no alterations in the total Chl and Chl a content; however, it resulted in an increased Chl a/b ratio and a decreased Chl b and Car content, as well as Chl fluorescence after 48 hours. These findings suggest that the volatile compound may have a detrimental effect on photosynthetic pigments. However, *C. comosum* may have modified its synthesis and functioning in order to protect its photosynthetic apparatus from HCHO-induced damage. This finding was corroborated with changes of the chlorophyll fluorescence kinetics, O-J-I-P test, through investigation into the parameters indicated a decrease in non-photochemical quenching (NPQ) in response to HCHO treatment, accompanied by an increase in various activity indices per reaction centre, including dissipated (DI0/RC), trapped (TR0/RC), and absorbed (ABS/RC) energy flux.

The functionality and structural efficiency of leaves of plants such as *C. comosum* L. are crucial to their ability to carry out photosynthesis, transpiration, detoxification of pollutants, and adaptation to environmental stress. It is evident that leaf structure, including chloroplast arrangement, mesophyll cell thickness and photosynthetic system efficiency, plays a pivotal role in ensuring that plants perform optimally under challenging environmental conditions, such as the presence of high formaldehyde concentrations in the air.

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Amaranth responses to heavy metal stress

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Keywords: grain amaranth, heavy metal, stress

Soil pollution with heavy metals is becoming one of the biggest environmental threats to crop production and could increase in near future. High metal concentrations in cultivated areas can significantly affect quality and yield of crops. Low productivity, stunted growth, a decline in nutritional quality and essential mineral content are the direct consequences of metal-induced plant stress and reduced soil fertility. The most dangerous are non-essential metals such as Cd, Pb, Hg and As, which are extremely toxic to plants even in low concentration. On the other hand, essential metals (Mn, Zn, Fe and Cu) are beneficial in low concentration but harmful in high concentration.

Several studies have investigated growth responses, transportability and accumulation of metals as well as the ability of *Amaranthus* spp. to phytoremediate. In the present study, three amaranth cultivars were tested in a hydroponic experiment under different concentrations of Cd, Pb, Zn and Mn. The responses of amaranth to heavy metals were examined at biochemical, physiological and molecular levels.

The preliminary results showed that the mechanisms of amelioration of heavy metal stress were cultivar dependent. Amaranth cultivars responded more strongly to Pb and Zn stress than to Cd and Mn, and the growth tolerance index decreased according to this pattern: Pb>Zn>Cd>Mn. Our results suggest a role of *Chit5* and *Ah24* in the response of amaranth to Cd stress. Overall, Plainsman appeared to be the most sensitive to induced heavy metal stress among the selected cultivars. The accumulation of the tested heavy metals in the above-ground parts of plant was not efficient, so that grain amaranth can be regarded as a phytotabilizator rather than a hyperaccumulator. However, translocation and accumulation of heavy metals in amaranth seeds needs to be investigated in future with regard to food safety.

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The roles of a conserved lncRNA family during heat stress response in angiosperms

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lncRNAs have been suggested to regulate diverse pathways during development and stress responses. Here we describe a family of long intergenic non-coding RNA (named as *HS-lncRNA1* and *2*) and investigate their functions during non-stress and abiotic stress conditions in *Arabidopsis*. We show that (i) the *HS-lncRNA1/2* are conserved in all examined angiosperm lineages and present in multiple copies in many species, (ii) they possess a highly conserved stem-loop structure, which likely comprise their functional unit, (iii) they are induced by heat, but not during cold, drought or salt stress; heat induces their transcriptional induction and post-transcriptional stabilization in parallel, (iv) HSFA1s and HSFA2 may be indirect negative regulators of their expression; and (v) HS-lncRNA1/2 are localized to the nucleus and strongly expressed in anthers and pollen grains.

To examine their specific requirement, we created single CRISPR mutant, while generation of double mutants is ongoing. In parallel, we aim to uncover their interactors and molecular partners.

In summary, HS-lncRNA family is possibly a central regulator of the double fertilization process off angiosperms during heat stress response.

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Plant spectral analysis for conservation habitat monitoring: Assessing of the endangered plant population stress status

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Keywords: conservation, leaf spectroscopy, endangered populations

The *Plantago maxima* Juss. ex Jacq. is a highly endangered perennial plant species in Hungary. In our country, its distribution reaches the western limit of its overall range. Presently documented occurences of the species are located within the areas of the Duna–Ipoly National Park and the Kiskunság National Park. These edge populations are extremely sensitive to environmental extremes. We lack detailed information about the plant physiological and environmental conditions of the Hungarian *Plantago maxima* populations, particularly regarding habitat soil characteristics.

Our study was conducted in 2024 on four isolated and fragmented populations of *Plantago maxima* in *Molinia* fen meadow and forest edge habitats in Kunpeszér, Tatárszentgyörgy, and Kakucs. At each site, we examined 15 flowering individuals, assessing their morphological and growth parameters (number and size of leaves and plant height). We also analyzed the chlorophyll content (CCI) and chlorophyll fluorescence (Fv/Fm) of the youngest fully developed leaf, as well as the leaf spectral characteristics (ARI, Ctr, CRI, FRI, NDVI, MCARI, NPCI, NPQI, PRI, RENDVI, SIPI, SPRI, VREI, WBI). The environmental conditions of the populations were evaluated based on the chemical and physical properties of the soil (soil EC, pH, soil water content, and concentrations of micro- and macronutrients).

The soil texture (K_A), pH, $CaCO_3$ content (%), and phosphorus concentration (P_2O_5 mg kg^{-1}) of the habitats varied within a narrow range. In the Kakucs soils, we measured significantly lower soil organic matter (humus %) and soluble mineral nitrogen content (NO_2+NO_3-N; mg kg^{-1}), while potassium (K_2O mg kg^{-1}) concentration was the highest. Due to the proximity of woody plants and their substantial water uptake through their large root system, soil water content was the lowest in the forest edge area at Kunpeszér. Leaf chlorophyll

content was not influenced by the habitat, whereas the Fv/Fm value was lowest at Tatárszentgyörgy. Depending on the habitat, the physiological parameters showed a strong correlation with soil water content and electrical conductivity (EC). The morphological indicators of the *Plantago maxima* individuals showed a strong correlation with SPRI, NPQI, Ctr, and ARI values, while Fv/Fm and CCI were closely associated with Ctr, ARI, WBI, and FRI indices. Based on leaf spectral and morphological data, the condition of the populations living near the forest of Kunpeszér and Tatárszentgyörgy was the poorest. Based on PCA analysis, the forest edge population at Kunpeszér was separated from the other habitats.

Leaf spectral data in response to stressors can drive a wide variety of insights into *Plantago maxima* health. Investigating the relationships between habitat environmental indicators, plant growth indices, and physiological parameters can support the identification of suitable habitats for new *in situ* populations. Our results may also aid in selecting optimal sites for establishing *ex situ* populations and in evaluating existing *ex situ* populations.

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Optimization of N and S fertilizations of oilseed rape for driving seed quality

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Keywords: Sulfur/nitrogen, seed quality, oilseed rape

Oilseed rape (*Brassica napus*) is a major oleoproteaginous crop used now for the edible oil market or as a protein source for animal nutrition as meals. In oilseed rape, nitrogen (N) and sulfur (S) nutrition together with genotype variability strongly impact seed yield and quality (fatty acids, proteins, micronutrients but also phytochemicals with antioxidant properties). Then, adjustments of N and S fertilizations of oilseed rape may lead to high seed yield and quality in a sustainable manner.

To confirm these nutritional interactions, two contrasted cultivars for seed quality (ES-Mambo and Bonanza) have been cultivated in 1m^3 soil containers (soil and seeding density comparable to field conditions) under a tunnel with contrasted N and S supply. Status of plants has been monitored throughout the culture by non-destructive methods such as Multiplex (N status: NBI-Or), Rapidscan (N status and Vegetation Index: NDVI, NDRE), XRF (elemental status of leaves, especially S), Pocket Pea (Photosynthetic status: F0/Fm). Harvested seeds has been analyzed for determination of mineral composition (XRF, IRMS), protein quality and phytochemicals through metabolomic workflows and NIRS.

Our current results show different behavior between the two varieties especially on N and S management. Considering seed quality, Bonanza has a higher protein and glucosinolate content and a lower fatty acid content than Mambo. In addition, Bonanza has a significantly higher N and S content than Mambo. The characterization of the seeds of these two varieties will be completed by more precise analysis of the protein quality in term of napin/cruciferin ratio (SDS-PAGE) and glucosinolate composition of the seeds (UPLC-MSTQD).

Correlation of non-destructive methods with seeds quality parameters will allow the development of predictive indicators of nutritional seed quality. These indicators will allow to screen genotypes with high seed quality and to propose the more relevant balance N/S fertilizations.

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JMJ9 interacts with Di19-3 to modulate the ubiquitin–proteasome system in tomato under heat stress

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Keywords: heat stress, H4R3me2s, histone demethylase, Di19-3, ubiquitin–proteasome system, tomato

The reversible addition and removal of methyl groups on histones play a crucial role in modulating chromatin structure and gene expression. However, the role of histone arginine demethylases in plant stress responses remains unclear. In this study, we identified a Jumonji C-terminal (JmjC) domain-containing protein, JMJ9, as an H4R3me2s demethylase in tomato (*Solanum lycopersicum* L.) that positively regulated thermotolerance and the degradation of ubiquitinated proteins. JMJ9 interacted with the transcription factor Drought-induced protein 19-

3 (Di19-3) to enhance the transcription of several ubiquitin-proteasome system (UPS) related genes and 26S proteasome activity during heat stress. Moreover, the JMJ9-Di19-3 module activated UPS-related genes (*HAKAI*, *UBC8*, *UBC-like*, and *PSMB6*) by removing the repressive H4R3me2s mark from their promoters. Mutation of Di19-3 suppressed JMJ9-induced thermotolerance. These findings highlight a JMJ9-mediated signaling pathway and the critical role of Di19-3 in 26S proteasome activity, degradation of ubiquitinated proteins and enhanced plant thermotolerance.

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REDOX BIOLOGY

P-239

Agrobacterium tumefaciens versus *Rhizobium rhizogenes*: A comparison of two transformation techniques to introduce roGFP2 redox probe for monitoring redox potential in *Arabidopsis* roots

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Keywords: redox regulation, fluorescent redox probe, genetic transformation

Cellular redox homeostasis ensures the balance between reducing and oxidizing reactions within cells and regulates a plethora of biological responses and events, but its study has proven difficult over time. Glutathione (GSH) is considered the master regulator of cellular redox homeostasis, and its redox potential (E_{GSH}) correlates well with the redox state of plant cells. To obtain information about the cellular redox state, we introduced the redox sensitive green fluorescent protein construct containing a fusion product of the human glutaredoxin (GRX1-roGFP2), which allows *in vivo* imaging the changes of E_{GSH} with high sensitivity and temporal resolution. The fluorescent redox probe was introduced into *Arabidopsis thaliana* either by *Agrobacterium tumefaciens* or by *Rhizobium rhizogenes*-mediated transformation. Hairy root cultures, developed after *R. rhizogenes*-plant interaction, offer an excellent research tool for analyzing changes in redox homeostasis under both normal and stressful conditions. Our aim was to compare the usefulness of the two different transformation techniques in detecting redox potential changes. Using our optimized *R. rhizogenes*-mediated transformation protocol, approximately 80% of Arabidopsis seedlings developed transgenic fluorescent roots. The dynamic changes of E_{GSH} were measured in wild-type (WT, ecotype Col-0), glutathione reductase 1 *(gr1)*, and dehydroascorbate reductase 2 *(dhar2)* mutant plants via confocal microscopy over 60 min following treatment

with 150 mM NaCl or 300 mM mannitol, in both primary and adventitious root zones. The *gr1* and *dhar2* mutants showed more positive redox potential than WT plants, which could be elevated by stress treatments further. The application of the two distinct transformation techniques did not impact the temporal variations in the redox state, these represented a remarkably similar pattern of fluctuations. *In situ* visualization of superoxide anion using nitroblue tetrazolium (NBT) and detection of intracellular H_2O_2 through diaminobenzidine (DAB) staining confirmed that the two different transformation methods similarly affect the formation of reactive oxygen species in root cells. Transformation mediated by *R. rhizogenes* has proven to be a faster and more feasible method of genetic manipulation that offers a suitable alternative to the more time-consuming *A. tumefaciens*-mediated transformation to analyze redox changes in roots in response to abiotic stresses.

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Modification of the ROS landscape in the shoot apical meristem of *A. thaliana* during flowering and floral organogenesis

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Keywords: floral transition, reactive oxygen species, shoot apical meristem

The shoot apical meristem (SAM) ensures continuous production of aerial organs in plants and its maintenance is critical for shoot growth and development. The vegetative SAM is organized in different zones and sub-populations of meristematic cells can be distinguished: stem cells are located in the central zone (CZ) and their daughter cells are pushed away toward the peripheral zone (PZ) where faster cell division and leaf initiation occur. Several genes are differentially expressed in CZ and PZ, among which genes encoding proteins of reactive oxygen species (ROS) metabolism. It was then inferred that ROS are unevenly distributed in the SAM, the superoxide ion being more abundant in the CZ while hydrogen peroxide would accumulate in the PZ. How this "ROS landscape" interferes with the changes in gene expression that occur in the SAM at the transition to flowering is presently unknown. To address this question, transgenic plants of *Arabidopsis thaliana* that ectopically express different enzymes of ROS metabolism in the expression domains of genes involved in the transition to flowering are being generated. In this model plant, flowers are initiated in the PZ of the SAM while the CZ remains indeterminate; specific genes are thus expressed in these two domains. *In situ* hybridization allowed us to confirm the expression domains of the transgenes and phenotyping of the plants is ongoing.

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Aldehyde dehydrogenase as a metabolic sensor of nitroxyl in Arabidopsis

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Keywords: nitric oxide, nitroxyl, Arabidopsis thaliana, hypoxia

Although nitric oxide (NO) is well recognized as a signaling molecule engaged in development and stress responses in plants, there is still lack of functional information on its single electron reduced and protonated homologue - nitroxyl (HNO). The triatomic molecule has unique chemical characteristics enabling it easy to migrate in a cellular environment, potentially providing a signaling function. In previous study, we demonstrated that HNO is endogenously formed in Arabidopsis cells [1]; however, it can spontaneously dimerize to form nitrous oxide (N_2O), which makes it difficult to detect endogenous HNO in living cells. Therefore, the identification of biological targets modified *via* HNO in cells could serve as a functional fingerprint of the cellular HNO presence. Nitroxyl can react with many targets, including molecular oxygen, nitric oxide, nitrite, hydroxylamine, sulfite, thiosulfate, metalloproteins, metalloporphyrins, thiols, C- and S-nitroso compounds [2].

Aldehyde dehydrogenase (ALDH) belongs to thiol proteins, which are the most recognized biological targets of HNO in animals, and is involved in the NAD^+ or $NADP^+$ - dependent conversion of various aldehydes to

their nontoxic carboxylic acids. The *ALDH* gene(s) are highly conserved in eukaryotes; hence, ALDH could also be a possible metabolic sensor of HNO in plant cells. To test this assumption, the study used the model plant *Arabidopsis thaliana*. To provoke endogenous HNO formation in cells, Arabidopsis plants were subjected to hypoxia, a stress accompanied by a reductive environment offering excellent conditions for HNO bioavailability. Moreover, exogenous HNO in the form of the donor compound was applied. Gene expression analysis indicated that among all 16 *ALDHs*, *ALDH2B7*, *ALDH3H1*, and *ALDH5F1* are the most responsive genes to both hypoxia and exogenous HNO. To reveal the physiological consequence of the potential modification of ALDHs by HNO, the selected proteins were cloned and recombinantly expressed in *E. coli*. Next, the purified proteins were used to test the enzyme activity. Importantly, the ALDH(s) enzyme activity decreased in a manner dependent on the concentration of the HNO donor. In conclusion, the results indicate that the selected ALDHs can serve as potential sensors of HNO at molecular and metabolic levels, and our future experiments should recognize the biochemical nature of HNO-mediated regulation of ALDH in Arabidopsis.

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P-242

Redox regulation of the response to cadmium in wheat

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Keywords: ascorbate, glutathione, hydrogen-peroxide, sodium hydrogen sulphide, phytochelatins

Heavy metal pollution is a serious environmental problem that limits crop productivity and threatens human health. Cadmium (Cd) is one of the most toxic environmental pollutants in the atmosphere, soil and water. Redox regulation is essential to maintain the normal plant metabolism under stressful conditions. In addition, phytochelatins formed from glutathione detoxify Cd by its chelation, thus contribute to plant defence against Cd stress.

Our experiments aimed to investigate the effects of externally applied redox reagents on the internal redox system of wheat and its tolerance to Cd stress. One-week-old wheat seedlings were pretreated with 20 mM ascorbate (Asc), 20 mM H_2O_2 or 500 µM NaHS for 3 days. Then, the plants were treated with 50 µM Cd for 1 week.

The results obtained indicated that the different pretreatments had various effects on the redox system of wheat under Cd stress. Shoot and root weight and length were reduced by Cd treatment and the effect was enhanced by Asc and H_2O_2 pretreatments compared with the control. The amount of glutathione increased significantly in the Asc-pretreated plants after Cd treatment. H_2O_2 pretreatment decreased the glutathione level, while NaHS pretreatment had no effect compared to the plants treated with Cd alone. Phytochelatin levels increased in roots after Cd treatment, but they were lower in plants pretreated with Asc, H_2O_2 or NaHS than in plants treated with Cd alone. The Cd treatment resulted in a large accumulation of Cd in both shoots and roots. The pretreatments slightly reduced the Cd levels in the roots. Cd accumulation in the shoots was reduced by the Asc pretreatment, it was not affected by the NaHS pretreatment, while H_2O_2 pretreatment promoted it.

The applied pretreatments altered the internal redox state of wheat plants, which influenced the defence mechanisms against Cd stress. The Asc and H_2O_2 pretreatments enhanced the negative effects of Cd, while the NaHS pretreatment did not influence it.

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Redox signaling to chromatin during stress responses in plants

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GENERAL CONTROL NON-REPRESSIBLE 5 (GCN5) is a subunit of the evolutionary conserved Spt-Ada-Gcn5-acetyltransferase (SAGA) complex, which is involved in acetylation of lysine residue on histone H3. GCN5 regulates the expression of genes involved in development, and abiotic and biotic stress responses. In addition, GCN5 plays a crucial role in cell wall synthesis, including lignin deposition, which is linked to the response to salt stress. However, little is known about the molecular mechanism of the regulation of GCN5 activity in response salt stress. Peroxidases are important members of enzymatic antioxidant defense machinery, which regulate the redox balance of the cell and thereby affect the oxidation of phenolic compounds promoting the formation of lignin. We analysed the expression of peroxidases (PRX) under salt stress condition using qRTPCR approach. We have found PRX71 and PRX33 to be potential candidates for further investigation of their roles under salt stress conditions. In our study, we explore the role of GCN5 mediated regulation of lignin deposition via the regulation of *PRX* genes under salt stress.

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Redox-mediated changes in hormones and metabolites in maize seedlings

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Ascorbate, hydrogen peroxide, and hydrogen sulfide each influence cellular redox homeostasis through distinct mechanisms. This study aimed to evaluate their relative effects on the redox state and hormonal balance in maize seedlings by applying one oxidant (5 mM H_2O_2) and two reductants (5 mM ascorbate and 1 mM NaHS). Among these, NaHS notably enhanced the reduced-to-oxidized glutathione ratio in the shoots, indicating a shift toward a more reduced cellular state. Conversely, ascorbate treatment lowered this ratio, suggesting an induction of oxidative stress. Correspondingly, ascorbate caused the most pronounced increase in both electrolyte leakage and lipid peroxidation, confirming its oxidative effect. Enzymes involved in detoxifying H_2O_2 showed differential responses: activities of dehydroascorbate reductase, monodehydroascorbate reductase, and catalase were significantly elevated after 7 days of ascorbate exposure, whereas ascorbate peroxidase peaked following H_2O_2 treatment. In addition to ascorbate, H_2O_2 also triggered substantial elevations in stress-related hormones such as jasmonic acid, salicylic acid, and abscisic acid, unlike NaHS. These hormonal and redox changes were accompanied by a marked reduction in shoot biomass under ascorbate and H_2O_2 treatments, indicating inhibited growth due to oxidative stress. Notably, the accumulation of phenolics and flavonoids also differed across treatments, suggesting redox-sensitive regulation of their biosynthesis. Altogether, the coordinated changes in antioxidant enzymes, hormonal profiles, and growth parameters underscore the complex interplay between redox status and signaling networks. These findings highlight the central role of redox balance in orchestrating physiological responses and stress adaptation in plants, offering potential strategies to improve crop resilience and performance under stress conditions.

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P-245

Parental exposure to soil residues of glyphosate induces intergenerational effects - A case-study with tomato plants

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Keywords: abiotic stress, intergenerational effects, oxidative stress

While the application of pesticides stands as an effective method of safeguarding agricultural production rates, their environmental impacts are a matter of concern. Recent research from our team has shown that soil residues of the herbicide glyphosate (GLY) can impair plant growth and cause oxidative stress in non-target crops [1]. Yet, little is known about its long-term consequences and potential transgenerational effects. Thus, this study aimed to understand if GLY can induce intergenerational responses on crops, using tomato plants (*Solanum lycopersicum* L.) as a model. Briefly, seeds from parental (F0) plants exposed to increasing GLY concentrations (0, 2.5, and 5.0 mg/kg) were collected and used to evaluate F1 sensitivity and response to a subsequent exposure to the herbicide. For this, each seed set (F0_CTR, F0_GLY2.5, and F0_GLY5.0) was exposed to GLY (0, 1.25, and 2.5 mg/kg) for 30 days to analyze intergenerational effects on biometrical parameters, oxidative stress markers, and antioxidant system modulation. Although GLY affected shoot and root growth regardless of the seed set, plants from GLY-treated parents presented lower inhibition rates, particularly in roots exposed to the lowest concentration (1.25 mg/kg). Interestingly, GLY exposure only resulted in severe oxidative damage, reflected by increased H_2O_2 and lipid peroxidation values, in plants derived from CTR parents (F0_CTR), with their efforts to enhance the antioxidant efficiency – increased proline content, and enhanced activity of antioxidant enzymes – not being able to prevent redox disorders. On the contrary, F0_GLY2.5 and F0_GLY5.0 plants showed no signs of oxidative damage, followed by differential modulation of their antioxidant system, generally showing a lower need to boost the enzymatic antioxidant performance, except for catalase, whose activity increased in F0_GLY5.0 plants under 2.5 mg/kg GLY. Overall, the data demonstrated that GLY soil residues can induce intergenerational effects, as GLY-mediated impacts on F1 plants depended on parental exposure, providing a more solid basis for future risk assessments.

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P-246

Hairy root transformation using rhizobium rhizogenes as a tool for exploring redox-specific gene expression and function in Arabidopsis

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Keywords: redox regulation, gene cloning, salt stress

The generation and regeneration of transgenic plants is essential for plant biology research to investigate plant physiology, pathogen interactions, and gene function. However, producing stable transgenic plants is time-consuming, which can impede research progress. Composite plants, derived from *Rhizobium rhizogenes*-mediated transformation, consisting of wild-type shoots and transgenic roots, are alternative research tools that can facilitate functional analysis of genes of interest involved in root redox regulation and development. In this research, we aimed to provide a Gateway cloning-compatible *R. rhizogenes*-mediated transformation

system that facilitates fast and reliable cloning of genes of interest in *Arabidopsis thaliana* L. plants. In the cloning experiments, we selected and cloned some tomato (*Solanum lycopersicum* L.) genes (dehydroascorbate reductase *SlDHAR1*, glutathione reductase *SlGR1*, glutathione synthetase *SlGSH1*) into overexpression binary vectors. These genes encode key enzymes involved in plant redox regulation. Using our optimized *R. rhizogenes*-mediated transformation protocol, we successfully introduced and expressed these tomato genes in Arabidopsis roots. The success of the work was confirmed by the appearance of transgenic roots on the selective medium within two weeks of transformation, and expression of genes of interest was also confirmed by real-time qPCR. Overexpression of tomato genes significantly enhanced the plant vitality and decreased the total amount of reactive oxygen species (ROS) in the transformed Arabidopsis root cells under salt stress. We introduced the orthologous *SlDHAR1* and *SlGR1* genes in Arabidopsis *dhar2* and *gr1* mutants for functional analysis. To visualize the intracellular redox state of the roots, we used Arabidopsis mutants that stably expressed the redox-sensitive green fluorescent protein (GRX1-roGFP2), allowing us to visualize the glutathione redox potential (E_{GSH}). Microscopic measurements with roGFP2 confirmed the restoration of redox state in the mutant Arabidopsis lines. Elucidating the function of additional genes using *R. rhizogenes*-mediated transformation could provide a more comprehensive understanding of the intracellular biological processes and establish its potential application for molecular plant breeding.

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Cystatin-mediated enhancement of human epidermal growth factor bioproduction in plants

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Keywords: plant-based protein production, EGF stability, cysteine protease inhibition

Plant-based systems offer a cost-effective platform for producing pharmaceutical proteins, but challenges remain due to the instability and rapid degradation of certain proteins, such as epidermal growth factor (EGF). This study aimed to improve EGF accumulation by targeting its subcellular localization and stabilizing its structure. EGF was successfully targeted to the chloroplast and the endoplasmic reticulum (ER), with higher accumulation in the ER. However, EGF degradation remained substantial, unlike other proteins such as green fluorescent protein (GFP) and fibroblast growth factor 1 (FGF). Co-expression of the cysteine protease inhibitor SlCYS8 significantly enhanced EGF stability, and a fusion protein of EGF and SlCYS8 exhibited superior stability and accumulation. Furthermore, a fusion protein containing both EGF and SlCYS8 was purified using protein A affinity chromatography, yielding pure, functional EGF. The biological activity of plant-produced EGF (P EGF) was confirmed by assessing EGFR phosphorylation in HaCaT cells and performing wound-healing assays, where P EGF demonstrated greater efficacy than commercial EGF. These findings demonstrate the potential of plant-based systems to produce stable and functional EGF, highlighting SlCYS8 as an effective tool for enhancing protein stability and production.

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PGPR isolated from the root zone of salt-tolerant *Petrosimonia triandra* enhance stress tolerance in plants

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Unfavorable climate changes experienced in recent years, such as rising temperatures and decreasing precipitation, can lead dangerous consequences for most agriculturally utilized soils and cause salinization and salt accumulation. Increased salinity limits the ability of plants to absorb water and nutrients, which can result in a drastic reduction in yield.

While one of the aims of plant breeding and biotechnology programs is to develop more stress-tolerant plant varieties, alternative strategies can also be developed to reduce these adverse effects. In nature, plants also live on water-deficient, salty soils. These salt-tolerant plants have developed several strategies to survive. One possible mechanism is the formation of a community between their roots and salt-tolerant bacteria found in saline soils. Bacteria that inhabit the root zone of salt-tolerant plants and provide beneficial effects are collectively referred to as plant growth-promoting rhizobacteria (PGPR). Many PGPR strains enhance plant growth by increasing salt or drought tolerance, promoting root development, or stimulating the production of osmoprotectants and protective proteins.

During our experiments, we isolated several bacterial strains from the root zone of the halophytic *Petrosimonia triandra*, which occurs on saline soils in Cluj County, Romania. These bacterial strains are able to grow on medium supplemented with 2 M NaCl. We identified the bacteria based on 16S rRNA gene sequencing, and tested their plant growth-promoting effects using *Arabidopsis thaliana* and *Brassica napus* (rapeseed). Several bacterial strains were found to promote Arabidopsis root and shoot growth in *in vitro* experiments using 125 mM NaCl containing medium. Additionally, we tested the root and shoot development of rapeseed plants in greenhouse experiments and we found that some bacterial strains enhanced plant resistance under salt stress, leading to better growth and development. Based on our preliminary data, our results suggest that the PGPR strains we isolated improved the salt tolerance of both Arabidopsis and rapeseed plants.

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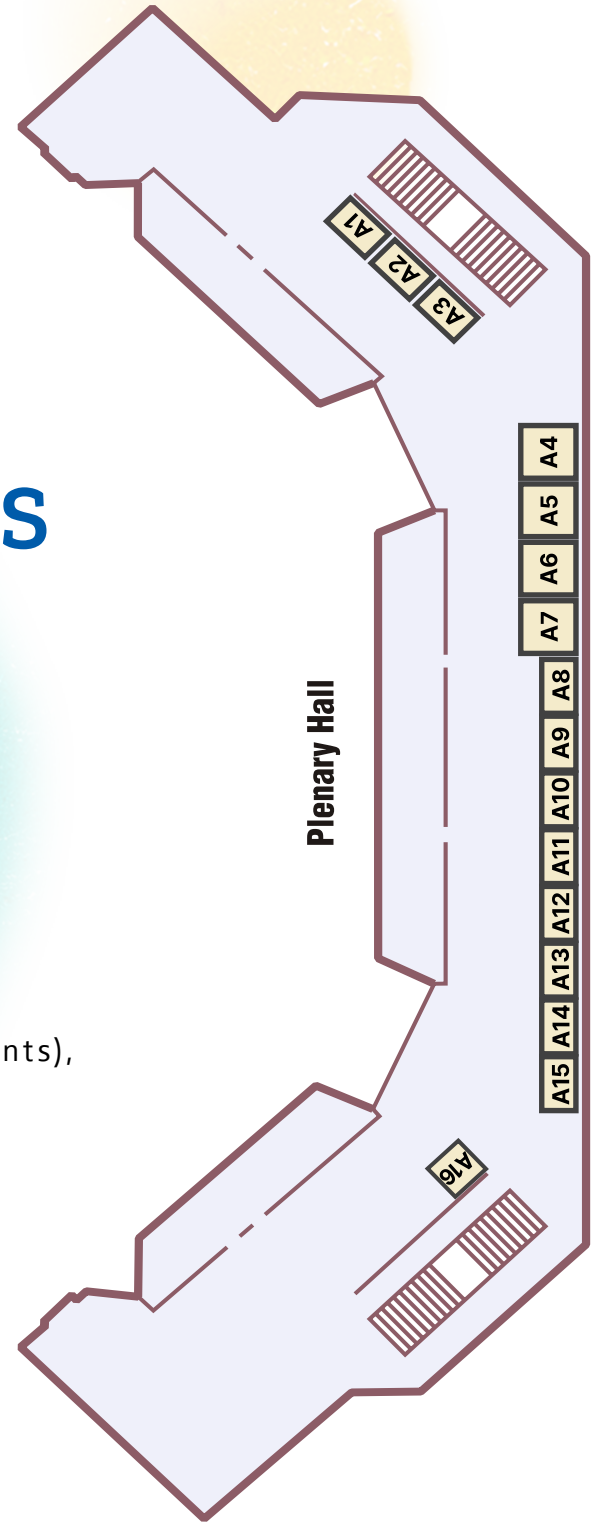

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