10th Plant Nitric Oxide International Meeting

Book of Abstracts

Warsaw & online
2025

Welcome message

Dear Participants,

It is our great pleasure to welcome you to the **10th Plant Nitric Oxide International Meeting**. This abstract book reflects our community's scientific breadth and diversity, showcasing current advances and new perspectives in the study of reactive nitrogen species—particularly nitric oxide—in plant systems.

The contributions gathered here represent the work of researchers from around the world, including many early-career scientists whose voices and ideas are vital to the future of this field. We hope this book's content will inspire engaging discussions, spark new collaborations, and foster further discovery.

We wish you a stimulating and fruitful meeting.

With kind regards,

Urszula Krasuska

Agnieszka Gniazdowska

Katarzyna Ciacka

Paweł Staszek

Agnieszka Wal

Marcin Tyminski

Maciej Piekarniak

Chrystian Chomontowski

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Technical guidelines

The conference will be conducted virtually via the Zoom platform.

We strongly recommend that all participants download and install the desktop version of the Zoom application.

Kindly verify your system configuration prior to the conference to ensure seamless screen sharing capabilities.

The poster session will take place in Zoom breakout rooms. Each presenter will be assigned their own room, where other conference participants can join to view the poster and engage in discussion.

Conference Programme

Please note that all timelines are given in Central European Time (CEST, UTC+02:00)

Wednesday 9th July 2025

13:00-13:10	Urszula Krasuska	**Start of the conference and welcome of the guests**
13:10-13:20	Urszula Krasuska	**Presentation about Warsaw and Warsaw University of Life Sciences (SGGW)**
Chair: Urszula Krasuska		
13:20-13:50	John Hancock	History of "The NO Club" or how did we get to PNO10?
13:50-14:30	Francisco J Corpas	Plant NO enzymatic production: An updated view
14:30-14:50	Break	
Reactive nitrogen species and metabolism Part I **Chairs: Zsuzsanna Kolbert Ördögné and Alexandre Boscari**		
14:50-15:30	Christian Lindermayr	Function of *S*-nitrosoglutathione reductase in adaptation to a changing environment
15:30-15:50	Ignacio Bienaime-Estevez	Redox regulation of the Primary Nitrate Response in plants
15:50-16:10	Priyanka Babuta	Recent advancements in the detection of reactive oxygen/nitrogen species in plant biology
16:10-16:30	Maria Meloni	Molecular and structural basis for nitrosoglutathione-dependent redox regulation of triosephosphate isomerase from *Chlamydomonas reinhardtii*
16:30-16:50	Agnieszka Wal	NO regulates the uptake of N and P in the pitcher trap of *Nepenthes x ventrata*

Please note that all timelines are given in Central European Time (CEST, UTC+02:00)

Thursday 10th July 2025

Reactive nitrogen species and metabolism Part II Chairs: Agnieszka Gniazdowska-Piekarska and Francisco J Corpas		
9:00-9:40	John T Hancock	The interactions of nitric oxide with intracellular redox status and the influence of molecular hydrogen
9:40-10:00	Lorena Aranda-Caño	Signalling and biodistribution of nitro-fatty acids in plant cells
10:00-10:20	Jorge Taboada	Polyamine oxidase (PAO) genes expression is differentially modulated by NO and melatonin in sweet and hot pepper (*Capsicum annuum* L.) fruits
10:20-10:40	Maciej Piekarniak	The impact of hydrogen cyanide on RNS content in apple (*Malus domestica* Borkh.) embryonic axes during seed dormancy alleviation
10:40-11:00	**Break**	
Reactive nitrogen species in abiotic stress Part I Chairs: John T Hancock and Marek Petřivalský		
11:00-11:40	Kapuganti Jagadis Gupta	The role of phytoglobin-nitric oxide cycle in low oxygen stress tolerance and nitrogen use efficiency
11:40-12:00	Shuhua Zhu	Effect of taurine treatment on nitric oxide metabolism of fresh-cut peaches
12:00-12:20	Soumya Mukherjee	NO, H_2S, and H_2O_2 crosstalk in abiotic stress: Cue to long-distance signaling
12:20-12:40	Ginevra M E Peppi	Structural and functional dissection of catalytic and redox properties of AKR4C isoforms from *Arabidopsis thaliana*
12:40-13:00	Diego Piacentini	The role of *Arabidopsis* CATALASE2 in root development involves NO and is hidden by cadmium treatment
13:00-13:20	**Break**	
13:20-14:20	**Poster Session**	
14:20-15:00	**Lunch Break**	

Please note that all timelines are given in Central European Time (CET, UTC+02:00)

Thursday 10th July 2025

Reactive nitrogen species in abiotic stress Part II Chairs: Magdalena Arasimowicz-Jelonek and Christian Lindermayr		
15:00-15:20	Rafael C da Silva	Nitric oxide-releasing chitosan nanoparticles improve water deficit tolerance in *Araucaria angustifolia* (Bertol.) Kuntze (Araucariaceae) seedlings
15:20-15:40	Patricia J Lopes-Oliveira	Influence of SlGSNOR manipulation on tomato responses to long-term moderately high temperature
15:40-16:00	Iara Deluca	Ultraviolet-B radiation induces NO accumulation and reduces biofilm formation in the cyanobacterium *Synechococcus* PCC 7335 *via* a NOS- and NR-independent mechanism
Reactive nitrogen species in biotic stress Part I Chairs: Christian Lindermayr and Magdalena Arasimowicz-Jelonek		
16:00-16:40	Marek Petrivalsky	Decoding nitric oxide signals: The *S*-denitrosation machinery in plants
16:40-17:00	Justyna Nawrocka	Nitric oxide signaling in management of tomato plant response to grey mold disease caused by *Botrytis cinerea*
17:00-17:20	Jakub Graska	The effects of soil salinity and the mite *Aceria tosichella* infestation on nitric oxide metabolism in barley

Please note that all timelines are given in Central European Time (CEST, UTC+02:00)

Friday 11th July 2025

Reactive nitrogen species in growth and development Part I Chairs: Jagadis Gupta Kapuganti and José Manuel Palma		
9:00-9:40	Alexandre Boscari	Nitric oxide homeostasis during legume–rhizobium symbiosis: balancing signaling, metabolism, and stress adaptation
9:40-10:00	Cylia Salima Oulebsir	Decoding the role of nitric oxide in pollen development
10:00-10:20	Miriam Molina-Escobar	Regulation by NO and ripening of genes involved in carotenoid biosynthesis in sweet pepper (*Capsicum annuum* L.) fruits
10:20-10:40	Marcin Tyminski	Nitric oxide impacts the proteome in embryonic axes of artificially aged apple (*Malus domestica* Borkh.) seeds
10:40-11:00	**Break**	
Reactive nitrogen species in growth and development Part II Chairs: Juan Barroso and John Hancock		
11:00-11:40	Zsuzsanna Kolbert Ördögné	Plant naNObiology: Nitric oxide in plant-nanoparticle interactions
11:40-12:00	Talita S Amador	Seed treatment with nitric oxide-releasing nanoparticles as a sustainable strategy to improve soybean growth and development: From the laboratory to the field
12:00-12:20	João Pedro C Pereira	Seed priming with nitric oxide-releasing nanoparticles as a strategy to enhance maize performance under field conditions
12:20-12:40	José López-Bucio	A quorum-sensing mutant of *Pseudomonas aeruginosa* promotes plant growth *via* nitrate reduction and transport and nitric oxide accumulation in roots
12:40-12:45	**Announcement of PNO11**	
12:45-13:15	**Poster session (additional)**	
13:15-13:45	**Scientific Committee meeting**	
13:45-14:00	**Conference Conclusion and Presentation of Awards**	

Poster index

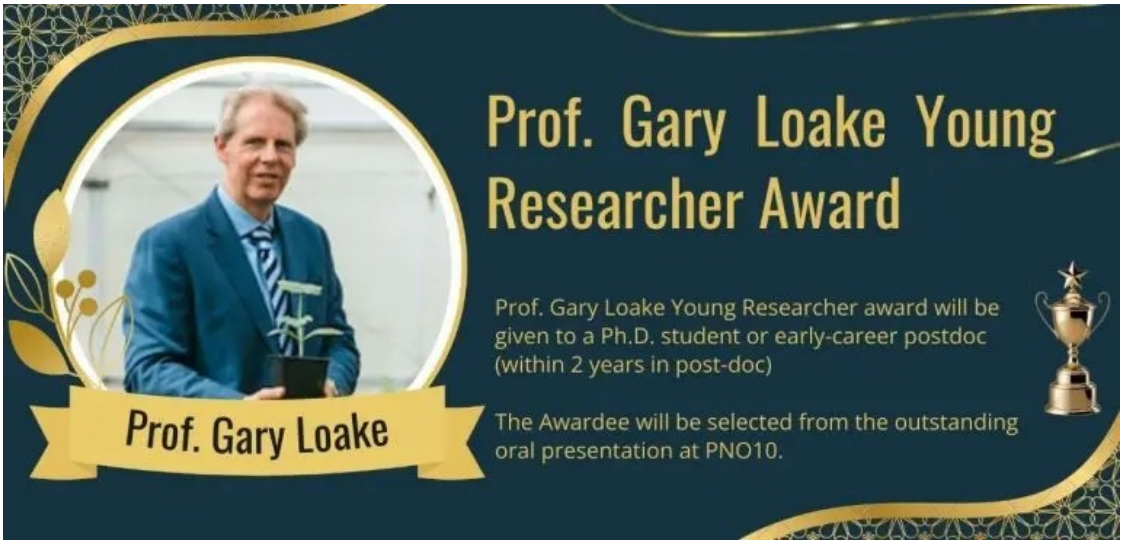
The numbers below indicate the breakout rooms where each poster will be presented during the poster session.

Breakout room no.	Presenter	Title
	Reactive nitrogen species and metabolism	
P1	Correa-Aragunde Natalia	Heterologous expression of an unusual NOS and its impact on nitrogen metabolism in cyanobacteria
P2	Demecsová Loriana	Nitric oxide and flavohemeproteins in barley root tips
P3	Gajewska Joanna	Copper-dependent nitro-oxidative stress modifies *Phytophthora infestans's* offensive strategy towards potato
P4	Kondak Dóra	Effect of chitosan-encapsulated *S*-nitrosoglutathione (GSNO) nanodonor on endogenous nitric oxide (NO) metabolism in *Brassica napus* seedlings
P5	Paluch-Lubawa Ewelina	Nitric oxide – polyamine cycle - hydrogen peroxide as the signaling triad regulating barley senescence
P6	Treffon Patrick	Protein-protein interactions of GSNOR in *Arabidopsis thaliana* reveal potential regulators of nitric oxide homeostasis
	Reactive nitrogen species in abiotic stress	
P7	Bodor Tamás	Impact of seed priming with plasma-activated water on physiologycal parameters of *Arabidopsis thaliana* L. seedlings
P8	Fejes Gábor	Seed pre-treatment with plasma-activated water influences plant development, reactive oxygen and nitrogen species, and photosynthetic performance under osmotic stress
P9	Guan Yufeng	Nitroxyl as a new regulator of hypoxia response in Arabidopsis
P10	Kleczkowska Wiktoria	NO/HNO balance and its anti-senescence potential in premature barley leaf senescence
P11	Montilla-Bascón Gracia	AsPgb1-mediated NO scavenging is associated with ethylene reduction and delayed drought-induced senescence in oat
P12	Silva Janaína	Nitric oxide-releasing nanoparticles as a tool for improving cotton drought tolerance
P13	Świercz-Pietrasiak Urszula	Effect of formaldehyde on *Chlorophytum comosum* and nitric oxide synthesis in plant cells
P14	Wróbel-Kwiatkowska Magdalena	Biological function of nitric oxide (NO) in stress response of transgenic flax (*Linum usitatissimum* L.) plants

Reactive nitrogen species in biotic stress		
P15	Sochańska Aleksandra	Spatio-temporal changes in the generation of nitric oxide during the interaction of tomato plants with the fungal pathogen *B. cinerea*
P16	Buet Agustina	GSNO treatment impacts on phytohormone levels in sweet cherry during storage
P17	Hong Jeum Kyu	Nitrate reductase-produced nitric oxide in Chinese cabbage leaves during the hypersensitive cell death against the non-adapted *Xanthomonas* bacteria
P18	Kozieł Edmund	Nitric oxide defensive potential in Arabidopsis -Turnip mosaic virus (TuMV) interaction
P19	Prats Elena	Unraveling the role of *S*-Nitrosylation during hypersensitive resistance to powdery mildew
Reactive nitrogen species in growth and development		
P20	Do Carmo Giovanna Camargo	Effect of *S*-nitrosoglutathione-containing chitosan nanoparticles on the development of *Cecropia pachystachya* seedlings under field conditions
P21	Maldonado Laura	Effects of seed priming with *S*-nitrosoglutathione-loaded chitosan nanoparticles on the early development of Atlantic Forest tree seedlings
P22	Stasolla Claudio	Transcriptome rewiring evoked by the intracellular location of Phytoglobin 2 during the induction of Arabidopsis somatic embryogenesis
P23	Széles Eszter	Optimization of *S*-nitrosoglutathione (GSNO) treatment for genetic screening of *Arabidopsis thaliana*
P24	Palma José Manuel	Variations in endogenous NO generation influence the peroxisomal and the redox metabolism in Arabidopsis leaves

Gary Loake

Young Researcher Award



Keynote speakers abstracts

History of "The NO Club" or How did we get to PNO10?

Hancock JT

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Now the Plant Nitric Oxide International Meeting (PNO) has reached its tenth conference it seems timely to review the journey of how we got here. PNO started as a "The NO Club", with its first meeting in Verona. It has moved around Europe, hosted by some of the most active research groups on the topic. More recently, partly driven by the COVID-19 pandemic, PNO went online, and with this could successfully move out of Europe for the first time. There have been sad losses along the way, but there have also been some significant outputs in the form of reviews and special issues in journals. PNO is now stronger and bigger than ever, and will no doubt help to encourage, facilitate and promote work on nitric oxide in plants well into the future.

Plant NO enzymatic production: An updated view

Corpas FJ

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Nitric oxide (NO) is a signaling molecule that plays a vital role in almost all physiological processes in plants. Although several enzymatic pathways for NO production in higher plants have been identified, particularly those involving L-arginine and nitrite [1,2,3], the precise sources of NO within specific subcellular compartments, organs, and physiological processes, especially under stress conditions, remain under active investigation and debate. This overview aims to provide an updated understanding of the enzymatic origins of NO in higher plants.

References:
[1] Corpas FJ *et al.* (2022) *Trends Plant Sci.* 27:116-119.
[2] Sedlářová M *et al.* (2025) *Int J Mol Sci.* 26:2087.
[3] Corpas FJ *et al.* (2025) *Plant Physiol Biochem.* 225:110000.

Acknowledgements:
Our research is supported by a European Regional Development Fund co-financed grants from the Ministry of Science and Innovation (PID2023-146153NB-C21 and CPP2021-008703), Spain.

Function of *S*-nitrosoglutathione reductase in adaptation to a changing environment

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The impact of climate change on plant life is difficult to predict. High levels of specific atmospheric compounds, changes in temperature and water deficiency are known to adversely affect plant physiology, partly by interfering with the plant redox system. However, the phenotypic and regulatory consequences of exposure to altered environmental conditions are largely unknown. Moreover, natural environmental conditions are challenging to simulate in the laboratory. Most environmental stress studies in plants have therefore probed only a few climate parameters in isolation and often only over a single generation. Insights from these studies are difficult to extrapolate to realistic climate conditions. We address these limitations and applied not only drought, heat, enhanced ozone and CO_2 separately, but also a complex, more natural climate scenario representing a combination of the single climate conditions, and grew *Arabidopsis thaliana* WT and *S*-nitrosoglutathione reductase knock-out plants. Whole genome bisulfite sequencing and transcriptome sequencing data revealed that plants grown under the different conditions showed both common responses, but also treatment-specific reactions. Interestingly, plants grown under heat and the complex climate scenario displayed similar DNA methylation and gene expression pattern. This experimental set-up promises to advance our basic understanding of how plant epigenome responds to such different climate scenarios, how this response affects plant performance and if *S*-nitrosoglutathione reductase is a key player at the interface between environment, plant epigenome and phenotypes.

The interactions of nitric oxide with intracellular redox status and the influence of molecular hydrogen

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Nitric oxide (NO) has a wide range of effects in both animals and plants. It will accumulate in cells, especially during stress responses, and lead to signalling events. Many of these downstream signals rely on *S*-nitrosylation of proteins, or nitration of proteins, but NO will also interact with a range of other cellular components, including lipids, but also other small reactive compounds. A well-known example of such a NO reaction is with the reactive oxygen species (ROS) superoxide, producing peroxynitrite. One characteristic of cells which is crucial to the control of cellular activity is the intracellular redox state, and this is maintained by compounds such as glutathione, but also impinged upon by ROS, reactive sulphur compounds such as hydrogen sulfide (H_2S), and potentially by hydrogen gas (H_2). Into this mix is NO, and here the potential influence of NO on cellular redox is discussed.

The role of phytoglobin-nitric oxide cycle in low oxygen stress tolerance and nitrogen use efficiency

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Plant encounter low oxygen stress due to flooding/waterlogging or during development of various organs of plants such as seeds. In the environment vast numbers of plant species are prone to flooding. In contrast, plants such as deepwater rice can withstand flooding and submergence. An important and interesting feature of rice is that it can germinate under anoxic conditions. Though several biochemical adaptive mechanisms play an important role in anaerobic germination of rice but the role of phytoglobin-nitric oxide cycle and mitochondrial alternative oxidase pathway is not known. Recently investigated the role of these pathways in anaerobic germination. Under anoxic conditions deepwater rice germinated significantly higher and rapidly than aerobic condition and the anaerobic germination and growth was much higher in the presence of nitrite which is intermediate of nitrate reduction reaction mediated by nitrate reductase. Addition of nitrite to germinating seeds stimulated NR activity and NO production. Important components of phytoglobin-NO cycle such as methaemoglobin reductase activity, expression of *Phytoglobin1, NIA1* and the promoters of these genes were elevated under anaerobic conditions in the presence of nitrite. The operation of phytoglobin-NO cycle also accelerated anaerobic ATP generation and fermentation metabolites such as lactic acid production and activity of ADH. Interestingly nitrite significantly reduced ROS production and lipid peroxidation. The reduction of ROS was accompanied by enhanced expression of mitochondrial alternative oxidase protein and its capacity. Operation of Pgb-NO cycle also improves nitrogen use efficiency via activation of HATs. Our study revealed that nitrite driven phytoglobin-NO cycle play role in hypoxia tolerance and nitrogen use efficiency.

Decoding nitric oxide signals: The *S*-denitrosation machinery in plants

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Within plant redox regulation, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved as signalling molecules or effectors of responses to external signals. Posttranslational modifications of proteins, such as cysteine *S*-nitrosation, are essential components of nitric oxide (NO) signalling. *S*-nitrosation regulates protein structure, functions and localisation, and is involved in the control of diverse cellular processes. Mechanisms of *S*-nitrosation and its effects on protein structures and activities have been described in detail in plants and other organisms; however, the precise site- and time-dependent regulation of *S*-nitrosation within the complex network of redox signalling is still not fully understood. The available data suggest that controlling levels of GSNO, the major low-molecular *S*-nitrosothiol, represents a primary mechanism regulating the dynamic processes of *S*-nitrosation and denitrosation, and hence the protein nitrosation status. GSNO is involved in transnitrosation reactions through which the NO group is transferred to the thiol group of another cysteine to form a new protein *S*-nitrosothiol. In plants, three key enzymes are involved in the regulation of *S*-nitrosothiols: *S*-nitrosoglutathione reductase (GSNOR), aldo-keto reductase (AKR), and thioredoxin (Trx) reductase (NTR). The balance between low-molecular-weight *S*-nitrosothiols and *S*-nitrosated proteins is indirectly controlled by NADH- or NADPH-dependent reductions of GSNO mediated by GSNOR and AKRs, respectively. The NADPH-dependent NTR/Trx system performs specific and efficient direct protein denitrosation and occupies a prominent role in multiple processes related to regulating protein structure and activity by modifications of protein cysteines. Elucidation of the molecular mechanisms of denitrosation in plants is important to understand how signal transduction pathways are regulated within plant metabolism during growth, development and stress responses.

Nitric oxide homeostasis during legume–rhizobium symbiosis: balancing signaling, metabolism, and stress adaptation

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Legume–rhizobium symbiosis leads to the formation of root nodules, where atmospheric nitrogen (N_2) is fixed by bacterial nitrogenase under microaerobic conditions. Nitric oxide (NO) accumulates at various stages of the symbiotic process, acting both as a signaling molecule and a metabolic intermediate. NO regulates gene expression, influences nodule development and senescence, and contributes to energy regeneration under hypoxia via the phytoglobin–NO respiration pathway. However, NO also inhibits nitrogenase and other key enzymes, making its tight regulation essential for efficient symbiotic functioning.

This balance is maintained by a coordinated network involving plant phytoglobins, bacterial hemoproteins, and nitrate reductases (NRs). In this study, we explore the early adaptive responses of *Medicago truncatula* roots and nodules in symbiosis with *Sinorhizobium meliloti* under short-term hypoxic stress induced by flooding. Particular attention is given to the regulation of phytoglobin–NO respiration in both roots and nodules and its contribution to energy metabolism and nitrogen fixation under low-oxygen conditions.

This presentation will offer an integrated view of NO dynamics and its regulation, emphasizing how NO homeostasis supports nodule resilience and metabolic flexibility under environmental stress.

Plant naNObiology: Nitric oxide in plant-nanoparticle interactions

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Nanomaterials include carbon-based and metallic nanoparticles (NPs) and due to their enhanced appearance in the environment the exposure of plants increases. Reactive nitrogen species (RNS) including nitric oxide (NO) are two-sided plant signal molecules, since they can mitigate or intensify stress damages in plants. Nanoparticle-associated phytotoxicity involves the accumulation and signalling of RNS/NO as has been demonstrated in our experiments with zinc oxide NPs, NiO NPs and multi-walled carbon nanotubes. The phytotoxicity proved to be dependent on plant species, ecotypes, NP concentration and size. It is well-researched that exogenous supplementation of NO may trigger plant tolerance against abiotic and biotic stressors. Our recent research focuses on nanoparticles delivering NO as novel ways of NO supplementation in stressed plants. For instance, chitosan encapsulated *S*-nitrosoglutatione (GSNO-CHT) has been applied in tomato plants for inducing defence against *Botrytis cinerea* infection, and fundamental mechanisms of NP internalization, NO liberation capacity and *in planta* NO signalling have been revealed in GSNO-CHT-treated oilseed rape seedlings.

Overall, this talk will give an overview about the known aspects of NO/RNS signalling in plant-nanoparticle interactions.

Acknowledgement:
The work was supported by the „Lendület" MOMENTUM project of the Hungarian Academy of Sciences (LP2023-14/2023) and the National Research, Development and Innovation Office (K146292).

Oral sessions abstracts

Session 1

Reactive nitrogen species and metabolism

Part I

Redox regulation of the Primary Nitrate Response in plants

Bienaime-Estevez I[1], Nejamkin A[1,2], Correa-Aragunde N[1], Scuffi D[3], Foresi N[1]

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Nitrogen (N) is an essential macronutrient for plant growth, being a key component in amino acids, proteins, and nucleic acids. Nitrate (NO_3^-) is the main N source for most crops. Its deficiency causes growth reduction and degradation of photosynthetic pigments, leading to chlorosis. Understanding how plants respond to NO_3^- is crucial, as large amounts of N are applied to boost productivity, but only 30–50% is retained in biomass. The rest is lost to water bodies, promoting eutrophication, leaches into groundwater, or is emitted as greenhouse gases. Although key molecular mechanisms of nitrate perception and physiological responses are known, many aspects remain unclear. We recently showed that nitric oxide (NO) is essential for the proper development of the Primary Nitrate Response (PNR). During PNR, there is a reduction in reactive oxygen species (ROS) and an increase in the activity of antioxidant enzymes like Catalase (CAT) and Ascorbate Peroxidase (APX). Hydrogen sulfide (H_2S), a gasotransmitter similar to NO, can modify cysteine residues through persulfidation, thereby affecting protein conformation and activity. In plants, H_2S is produced by several enzymes, including the cytosolic L-cysteine desulfhydrase 1 (DES1), which catalyzes L-cysteine degradation, yielding pyruvate, ammonium, and H_2S. DES1 contributes to 20% of total H_2S production. Our results suggest that H_2S modulates proteins post-translationally during PNR. We also showed that nitrate treatment alters the persulfidation protein profile. Different targets were identified, including antioxidant enzymes. These findings reveal new links between nutrient and redox signaling.

Recent advancements in the detection of reactive oxygen/nitrogen species in plant biology

Babuta P and Gupta KJ

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Reactive nitrogen species (RNS) and reactive oxygen species (ROS) have well-established significance in plant biology. Recent advancements in detecting RNS and ROS have enhanced our understanding of plant development and stress responses. These radicals have short lives and hence, need sophisticated detection methods. In the present study, we are discussing developed advanced tools and methodologies to detect these radicals simultaneously. One such chemiluminescence-based tool is CranoxII (EcoPhysics) allowing highly sensitive, real-time measurement of nitric oxide (NO) and other oxides of nitrogen (NOx). It is recommended to measure these radicals by at least two different methods to ensure accuracy. Hence, chemiluminescence data is complimented by advanced fluorescent probes (such as DAF-FM-DA for NO, H_2-DCF-DA for ROS, and MitoSOX for mitochondrial ROS), enabling precise detection. Additionally, the integration of proteomics to analyze *S*-nitrosylation and tyrosine nitration has provided deeper insights into the implication of RNS dynamics in cellular signaling. Since Internal oxygen level also plays a crucial role in radical production, we have optimized protocols to measure spatial and temporal oxygen dynamics in live plant tissue using invasive and non-invasive techniques. The present work will not only improve the fundamental understanding of plant physiology but also pave the way for practical applications in the path of sustainable agriculture.

Molecular and structural basis for nitrosoglutathione-dependent redox regulation of triosephosphate isomerase from *Chlamydomonas reinhardtii*

Meloni M[1], Mattioli EJ[2,3], Fanti S[2], Peppi GME[1], Bin T[1], Gabellini G[2], Tedesco D[4], Henri J[5,6], Trost P[1], Lemaire SD[5,6,7], Calvaresi M[2,3], Fermani S[2,8], and Zaffagnini M[1]

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Protein *S*-nitrosylation is a reversible redox-based post-translational modification that plays an important role in cell signaling by modulating protein function and stability. At the molecular level, *S*-nitrosylation consists of the formation of a nitrosothiol (-SNO) and is primarily induced by the trans-nitrosylating agent nitrosoglutathione (GSNO). Triosephosphate isomerase (TPI), which catalyzes the interconversion of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate, has been identified as a putative target of *S*-nitrosylation in both plant and non-plant systems [1,2,3]. Here we investigate the molecular basis for GSNO-dependent regulation of chloroplast TPI from the model green alga *Chlamydomonas reinhardtii* (CrTPI). Molecular modelling identified Cys14 and Cys219 as potential sites for interaction with GSNO, though crystallography of GSNO-treated CrTPI revealed *S*-nitrosylation only at Cys14. To disclose GSNO target sites, we generated and characterized Cys-to-Ser variants for Cys14 and Cys219, identifying Cys219 as a key residue mediating the GSNO-dependent modulation of CrTPI activity. Molecular dynamics simulations further revealed the stabilizing interactions of nitrosylated cysteines with their local environments. Overall, our results indicate that CrTPI catalysis is modulated by GSNO through a redox-based mechanism involving Cys219, highlighting a conserved regulatory strategy shared with human TPI.

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NO regulates the uptake of N and P in the pitcher trap of *Nepenthes x ventrata*

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Carnivorous plants attract animals, trap and kill them, then absorb nutrients from the digested bodies. This adaptation is crucial for their survival in low-nutrient environments, particularly those deficient in nitrogen (N) and phosphorus (P). Pitcher plants (*Nepenthes*) belong to the group of carnivorous plants that develop passive, pitcher-shaped traps at the end of the main vein of the leaf blade [1,4]. These organs are filled with digestive fluid, in which enzymes, reactive oxygen species (ROS), and reactive nitrogen species (RNS) have been identified [2,3].

The aim of this study was to investigate the role of NO in the regulation of N and P uptake in the pitcher trap of *Nepenthes* × *ventrata* during digestion. The material consisted of digestive fluid and trap tissue, which were collected 1, 2, and 4 days after introducing a protein source or a protein source supplemented with NO donor. Unfed traps were used as the control.

The measurement of activity of proteolytic and acid phosphatases, determination of transcript levels of genes encoding phosphatases and proteases (Nepenthesins), and measurement of the concentrations of P and NH_4^+ in the digestive fluid were done.

All studied parameters were stimulated after the addition of NO with the protein source, particularly in the initial stages of digestion. These findings indicate that NO may regulate the absorption of nutrients in *Nepenthes* × *ventrata*.

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Session 2

Reactive nitrogen species and metabolism

Part II

Signalling and biodistribution of nitro-fatty acids in plant cells

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Nitrated fatty acids (NO_2-FAs) are bioactive lipids formed by the reaction of unsaturated fatty acids with reactive nitrogen species (RNS) derived from nitric oxide (NO). In plants, the main NO_2-FAs identified are nitro-oleic (NO_2-OA), nitro-linoleic (NO_2-LA), nitro-conjugated linoleic (NO_2-cLA), and nitro-linolenic acid (NO_2-Ln). Their levels decrease during development but increase under abiotic stress [1,2]. NO_2-FAs can release NO, incorporate into complex lipids, and modify proteins via nitroalkylation of nucleophilic residues such as cysteine and histidine. They are stored in triglycerides and phytosterols in seeds, and in phospholipids during vegetative growth, where they associate with membranes, influencing structure, fluidity, and protein interactions [2,3]. Although their signalling pathways are still under investigation, NO_2-Ln has been shown to activate stress-responsive genes, including heat shock proteins [1]. Additionally, NO released from NO_2-FAs contributes to the formation of nitrosoglutathione (GSNO) and promotes the *S*-nitrosylation of GSNO reductase (GSNOR), modulating *S*-nitrosothiol metabolism [4,5]. NO_2-FAs also enhance seed germination by facilitating the *S*-nitrosylation of proteins such as ABI5, a repressor of germination, and by stabilizing the transcription factor bZIP67, which regulates fatty acid composition [5,6]. Furthermore, NO_2-FAs play a key role in oxidative stress responses through nitroalkylation, which reversibly inhibits antioxidant enzymes like ascorbate peroxidase (APX2) and catalase (CAT2) via adduct formation with catalytic residues. Under elevated reactive oxygen species (ROS) conditions, these adducts are oxidized, releasing NO_2-FAs and restoring enzymatic activity. This redox-sensitive mechanism positions nitroalkylation as a dynamic modulator of antioxidant defences [7,8]. Together, NO_2-FA act as crucial signalling molecules in plant development and adaptation to stress.

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Polyamine oxidase (PAO) genes expression is differentially modulated by NO and melatonin in sweet and hot pepper (*Capsicum annuum* L.) fruits

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Polyamines (PAs) are ubiquitous low molecular weight aliphatic compounds with two or more amino groups in their structure. These compounds are implicated in numerous physiological processes such as plant growth, flowering, fruit development, and ripening, among others. Also, PAs are correlated with the nitric oxide (NO) generation during plant development and under stress [1]. On the other hand, the PAs catabolism is mainly carried out by amino oxidases, highlighting the flavin-containing polyamine oxidases (PAO) that are a source of H_2O_2 by oxidizing spermine (Spm) and spermidine (Spd) to their acetylated derivatives. Melatonin in plants has gained the interest of many scientists due to its antioxidant and signaling properties in various plant processes [2,3]. However, there is limited information on how exogenous melatonin may influence the metabolism of fruits, particularly in pepper (*Capsicum annuum* L.) fruits. Pepper fruits, which have significant agroeconomic importance worldwide, are widely consumed both fresh and processed. Nutritionally, these fruits contain several compounds with antioxidant properties, including vitamin C, provitamin A, vitamin E, flavonoids, phenolics, and capsaicinoids, these latter particularly in hot varieties. Using sweet and hot pepper fruits exposed to NO and melatonin treatments and transcriptome analysis by RNA sequencing (RNA-seq), six genes designated as *CaPAO1* to *CaPAO6* were identified. The time-course expression analysis of these genes during the ripening process and due to the assayed treatments revealed differential modulation.

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Acknowledgements:
Our research is supported by European Regional Development Fund co-financed grants from the Ministry of Science and Innovation (PID2023-146153NB-C21 and CPP2021-008703), Spain.

The impact of hydrogen cyanide on RNS content in apple (*Malus domestica* Borkh.) embryonic axes during seed dormancy alleviation

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HCN is produced by approximately 3000 different plant species from over 130 plant families [1]. The activity of HCN has mainly been studied concerning response to biotic stressors [2,3,4] and regulation of seed dormancy and germination [5,6,7]. Although it has a "toxic face", HCN is increasingly recognisable as a signalling molecule in plants, playing a role in regulating various physiological processes like plant development, defence against pathogens, and response to environmental stresses. Due to its small molecular size and lipid solubility, HCN can easily permeate membranes and alter metabolism [8]. Reactive nitrogen species (RNS), which are derivatives of NO, play a crucial role in plant physiology. The role of RNS in seed physiology specifies the "nitrosative door" model, which reflects hypothetical concentrations of RNS that indicate a specific physiological function. According to this model, during seed dormancy, an increase in RNS content to the value referred to as "golden key" leads to seed germination ("opens the door"). Both HCN and NO are released from apple seeds during the cold stratification process, indicating their signalling role during dormancy alleviation. The aim of this work was to determine the impact of HCN on RNS content in apple (*Malus domestica* Borkh.) embryonic axes during dormancy alleviation. We determined the NO and peroxynitrite ($ONOO^-$) emission and NO_2^- concentration in embryonic axes isolated from apple embryos pre-treated with KCN (70 mM). The activity of *S*-nitrosoglutathione reductase (GSNOR) and the content of 3-nitrotyrosine (3-NT) in proteins were measured. Our data indicate that HCN treatment leads to the formation of 3-NT groups in proteins, stimulates the NO and $ONOO^-$ emission, increases NO_2^- concentration, and inhibits GSNOR activity in apple embryonic axes.

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Session 3

Reactive nitrogen species in abiotic stress

Part I

Effect of taurine treatment on nitric oxide metabolism of fresh-cut peaches

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The post-harvest preservation effect of taurine on fresh-cut peaches was investigated. Fresh-cut peaches were treated with a taurine aqueous solution, while the control experiment was soaked in distilled water. The taurine treatment alleviated the decline in firmness and the increase in color difference of fresh-cut peaches. The nitric oxide (NO) metabolism indices included endogenous nitric oxide (NO) content, *L*-arginine (*L*-Arg) content, nitric oxide synthase like (NOS-like) and nitrate reductase (NR) activities of fresh-cut peaches during storage were detected. Compared with control treatment, taurine treatment maintained high content of endogenous NO, enhanced *L*-Arg content and NOS-like activity, however the NR activity was inhibited in the first 12 days and then increased in the following days. The results indicated that the accumulated NO content in the first 12 days was mainly caused by NOS-like catalyzing *L*-Arg, and in the following 12 days was attributed to the combined action of NOS-like and NR. Therefore, taurine treatment might improve the storage quality by enhancing the endogenous NO metabolism in fresh-cut peaches.

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NO, H_2S, and H_2O_2 crosstalk in abiotic stress: Cue to long-distance signaling

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Among the various roles in plant systems, nitric oxide (NO) and hydrogen sulfide (H_2S) function as the two important gasotransmitters in regulating root development and aquaporin signaling. Hydrogen peroxide (H_2O_2) is a reactive oxygen species (ROS) and a key modulator of the development and architecture of the root system under physiological and challenging environments. However, the dose and mode of application of NO and H_2S can imply synergistic or antagonistic actions in mediating H_2O_2 signaling during root development. Thus, H_2O_2-NO-H_2S interaction is essential to elude oxidative stress in roots. Growth and proliferation of root apical meristem involve precise regulation of NO and H_2S-mediated ROS signaling which further involve other components including mitogen-activated protein kinase, cyclins, cyclin-dependent kinases, respiratory burst oxidase homolog (RBOH), and Ca^{2+} flux. Furthermore, the understanding of the mechanism of interactions of aquaporin with H_2O_2, NO and H_2S is obscure. Here, to investigate the crosstalk of aquaporin function, H_2O_2 homeostasis, and NO-H_2S signaling, we performed a computational prediction of cysteine-based oxidative post-translational modifications (oxiPTMs) of various aquaporin proteins in plants. We propose that aquaporin activity could be regulated by three major oxiPTMs, *S*-nitrosation, *S*-sulfenylation, and persulfidation, mediated by NO, H_2O_2, and H_2S, respectively. Therefore, aquaporins might be key players in the gasotransmitter-mediated long-distance oxidative stress signals in plant cells.

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Structural and functional dissection of catalytic and redox properties of AKR4C isoforms from *Arabidopsis thaliana*

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S-nitrosoglutathione (GSNO), an intracellular reservoir of nitric oxide (NO), plays a key role in its transport and biological activity. Although GSNO homeostasis is primarily regulated by the ubiquitous NADH-dependent *S*-nitrosoglutathione reductase (GSNOR), aldo-keto reductases of the subfamily 4C (AKR4Cs) have also been identified as alternative enzymatic systems that can cooperate with GSNOR in controlling GSNO levels. In this study, the biochemical features of *A. thaliana* AKR4Cs (isoforms C8-C11) were characterized, with a focus on their kinetic properties and redox sensitivity. These analyses revealed that AKR4C8 is the isoform that mostly resembles GSNOR in terms of substrate/cofactor affinity and redox sensitivity. Using site-directed mutagenesis and heterologous expression of recombinant AKR4C8 forms, we examined the relevance of the catalytic tetrad (Asp43, Tyr48, Lys77 and His117) demonstrating that each residue is essential for NADPH-dependent GSNO degradation. Structural analyses confirmed that the native folding was preserved in all mutants, ruling out any structural alterations caused by the mutations. The redox sensitivity of AKR4C8 was investigated by measuring enzyme activity following treatment with oxidizing molecules such as oxidized glutathione, hydrogen peroxide and GSNO. Based on available crystallographic data, solvent accessibility analysis of cysteine residues identified Cys287 as the most likely candidate for oxidation. Substitution of Cys287 with alanine didn't affect specific activity or native folding but rendered the variant completely insensitive to oxidation, indicating that Cys287 is the solely responsible for the redox sensitivity of AKR4C8. Overall, our results establish that specific residues within AKR4C8 are critical for both catalytic activity and redox regulation. Ongoing investigations, including phenotypical analysis of *knock-out* Arabidopsis plants, will offer further insight into the functional role and physiological relevance of AKR4C8.

The role of *Arabidopsis* CATALASE2 in root development involves NO and is hidden by cadmium treatment

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Heavy metal stress in plants is particularly concerning due to its ability to induce nitro-oxidative alterations in plant tissues. To mitigate these effects, plants can rely on an antioxidant system composed of both enzymatic and non-enzymatic molecules. Catalase (CAT) is a key antioxidant enzyme that catalyses the dismutation of H_2O_2 into H_2O and O_2 [1]. In plants, CAT is encoded by small gene families that produce multiple isozymes, with *Arabidopsis thaliana* possessing three CAT genes. *CAT1* is mainly expressed in pollen and seeds; *CAT2* is the primary photorespiratory isoform and is largely found in photosynthetic tissues and to a lesser extent in roots and seeds; while *CAT3* is found in vascular tissues and senescent leaves [2]. Although the role of CAT in plant development and stress responses is well documented, most studies focus on its function only in photosynthetic tissues. As a result, changes in CAT activity observed in roots are often considered indirect effects of alterations occurring in the aerial parts of the plant. In this study, we employed the *cat2-1* mutant to investigate the role of the CAT2 isoform in the root system of *Arabidopsis thaliana* after exposure to cadmium (Cd) stress. Immunodetection of root catalase confirmed the absence of CAT2 isoform in *cat2-1* roots and an increase in CAT1 and CAT3 isoforms in both *cat2-1* and *wild-type* genotypes upon Cd exposure. Also, *cat2-1* exhibited a shorter primary root and a reduced number of mature lateral roots compared to the wild type. Histological analysis suggests that the lower number of observed lateral roots may be due to impaired development caused cellular damages, likely resulting from increased production of reactive nitrogen species, particularly NO. However, the effects of the *cat2-1* mutation on the root system are hidden when plants are grown in the presence of Cd, revealing a role for this isoform in root development under physiological conditions.

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Session 4

Reactive nitrogen species in abiotic stress

Part II

Nitric oxide-releasing chitosan nanoparticles improve water deficit tolerance in *Araucaria angustifolia* (Bertol.) Kuntze (*Araucariaceae*) seedlings

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Global warming and reduced water availability pose serious threats to the survival and regeneration of *Araucaria angustifolia*, a critically endangered Brazilian conifer. As water deficit (WD) becomes more frequent under climate change, effective strategies to enhance seedling tolerance are essential. One promising approach involves chitosan-based nanoparticles releasing nitric oxide (NO), a signaling molecule involved in plant growth, development, and particularly in responses to abiotic stress. Building on this, this study investigated the protective effects of nanoencapsulated *S*-nitrosoglutathione (GSNO), an NO donor, in *A. angustifolia* under WD. Seedlings were grown under well-watered and WD conditions and treated with nanoparticles containing GSNO or glutathione (GSH). Morphological, physiological, and biochemical traits were assessed, including shoot and root growth, dry matter, water status, oxidative stress markers (MDA, H_2O_2), antioxidant enzyme activities, total soluble sugars (TSS), starch, proteins, and metabolic profiles. Results demonstrated that GSNO treatment conferred significant protective effects under WD. It reduced oxidative stress by enhancing antioxidant defenses, lowering reactive oxygen species, and increasing S-nitrosothiol content. Furthermore, GSNO promoted osmoprotectant synthesis, notably elevating TSS levels, which supported osmotic balance and water retention. Although WD negatively impacted growth, GSNO mitigated these effects, particularly promoting shoot development. Some similar effects observed with GSH-loaded nanoparticles suggest that GSH may also stem from GSH's antioxidant properties. However, the main protective results were observed in plants that received GSNO-loaded nanoparticles. In conclusion, nanoencapsulated GSNO alleviated WD-induced damage and enhanced seedling performance, offering a promising tool for improving drought tolerance in conifers and supporting conservation and reforestation efforts under climate stress.

Acknowledgments:
This study was funded by the São Paulo Research Foundation (FAPESP), the National Council for Scientific and Technological Development (CNPq), and the Araucaria Foundation (Fundação Araucária) in partnership with the Paraná State Secretariat of Science, Technology, and Higher Education (SETI) through the NAPI Emergência Climática initiative.

Influence of SlGSNOR manipulation on tomato responses to long-term moderately high temperature

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Improving plant thermotolerance is essential to ensure crop productivity under climate change scenarios. High temperatures negatively impact both vegetative growth and reproductive development, compromising photosynthesis, water status, and fruit production. Nitric oxide (NO) plays a key role in plant responses to heat stress, and *S*-nitrosoglutathione reductase (GSNOR) regulates NO/*S*-nitrosothiol homeostasis. Here, we investigated the effects of SlGSNOR overexpression (*SlGSNOR-OE*) and knockout (*Slgsnor*) in tomato (*Solanum lycopersicum*) plants under prolonged moderate heat stress (32 °C/25 °C day/night), compared to control conditions (25 °C/18 °C). Under heat stress, most photosynthetic parameters declined across genotypes, except for electron transport rate (ETR) and maximum quantum yield of photosystem II (*Fv/Fm*). Interestingly, *Slgsnor* mutants displayed higher net photosynthesis, carboxylation efficiency, and water-use efficiency compared to wild-type (WT), despite elevated hydrogen peroxide (H_2O_2) levels, without evidence of increased oxidative damage. In contrast, *SlGSNOR-OE* plants showed a clear advantage in fruit productivity under heat, with increased fruit set and harvest index, producing more and larger fruits than WT. Notably, this improvement was not associated with higher flower or inflorescence production, indicating more efficient resource allocation toward fruit development. Conversely, *Slgsnor* mutants exhibited severely reduced fruit set and smaller fruits under heat stress, despite normal flower production, suggesting impaired reproductive success under high temperatures. These findings highlight the critical role of SlGSNOR in regulating vegetative and reproductive performance under heat stress and support its biotechnological potential for developing climate-resilient tomato cultivars.

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Ultraviolet-B radiation induces NO accumulation and reduces biofilm formation in the cyanobacterium *Synechococcus* PCC 7335 *via* a NOS- and NR-independent mechanism

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Nitric oxide (NO) is a gaseous molecule involved in the protection of photosynthetic organisms against ultraviolet-B radiation (UV-B). This role is associated with the enzymatic production of NO through nitrate reductase (NR) in plants and nitric oxide synthases (NOS) in cyanobacteria. A non-canonical NOS in the cyanobacterium *Synechococcus* PCC 7335 metabolizes arginine producing 25% NO and 75% nitrate. *S.* PCC 7335 exhibits several adaptations to varying light regimes. The aim of this study was to analyze the role of NO in the response to UV-B in *S.* PCC 7335. Results showed that UV-B delayed *S.* PCC 7335 growth without loss of viability and increased NO levels compared to control. However, the addition of cPTIO had no effect on growth either in control or UV-B exposed cultures. To elucidate the pathway of NO production during irradiation, *in vitro* NOS activity using the Griess reaction and transcript level quantification using RT-qPCR were measured. Results showed that UV-B did not alter NOS activity and *NOS* transcripts were downregulated being unaffected by cPTIO treatment. The evaluation of a non-specific NR inhibitor on NO levels showed that had no effect during UV-B. Also, *NR* transcripts were downregulated by this radiation and were not regulated by endogenous NO. *S.* PCC 7335 formed a biofilm as evidenced by the crystal violet assay. UV-B decreased its formation, while the planktonic cell population remained unchanged. Co-treatment with UV-B and cPTIO, showed a slight reversal of the UV-B effect over biofilm formation. Accordingly, treatment with the NO donor SNP resulted in decreased biofilm formation. In conclusion, UV-B induces a deregulated increase in NO that is independent of NOS and NR activity which is associated with a reduction in biofilm formation in *S.* PCC 7335. This study contributes to the general knowledge of cyanobacteria responses to UV-B radiation and NO role, which in *S.* PCC 7335 differs from those described for other cyanobacteria.

Acknowledgements:
Supported by Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT), CONICET and Universidad Nacional de Mar del Plata.

Session 5

Reactive nitrogen species in biotic stress

Nitric oxide signaling in management of tomato plant response to grey mold disease caused by *Botrytis cinerea*

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Trichoderma is a fungal genus of interest to agriculture as it includes species that act as biological control agents (BCAs) of phytopathogens. Some *Trichoderma* strains are able to activate a type of resistance called *Trichoderma*-induced systemic resistance (TISR) which integrates signaling pathways that protect plants against both biotrophic and necrotrophic pathogens. Some studies suggest that reactive nitrogen species (RNS), particularly nitric oxide (NO), may play a significant role in TISR against various pathogens, including *Botrytis cinerea*. The present studies show that *Trichoderma virens* TRS 106 reduces grey mold disease caused by *B. cinerea* in tomato plants (*Solanum lycopersicum* L.) by enhancing their defence responses. Histochemical analyses revealed that *B. cinerea* infection caused NO accumulation in chloroplasts, which was undetectable in plants treated with TRS 106. Treatment of plants with TRS 106, however, caused systemic spreading of hydrogen peroxide (H_2O_2) and NO in the apoplast and nuclei. SPME GCxGC TOF-MS analysis revealed 24 volatile organic compounds (VOCs) released by tomato plants treated with TRS 106. These included hexanol derivatives such as 4-ethyl-2-hexanal and 1,5-hexadien-3-ol, as well as salicylic acid derivatives such as 4-heptanal and isoamyl salicylate. These compounds are considered to play a role in protecting tomato plants against *B. cinerea* for the first time. These results are valuable for further studies investigating the location and function of NO in plants treated with *Trichoderma*, as well as for verifying the contribution of the detected VOCs to plant protection against *B. cinerea*.

Acknowledgement:
This work was supported by the National Science Centre, Poland, by the grant „Intense smell in organic farming - the endogenous nitric oxide and sulfur-containing volatile compounds in the protection of tomato plants against gray mold disease, based on the use of microbiological biocontrol agents" (Grant no. 2023/51/D/NZ9/01972).

The effects of soil salinity and the mite *Aceria tosichella* infestation on nitric oxide metabolism in barley

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The primary nitrogen source for plants is nitrate ions (NO_3^-), which are taken up via active transport. Nitrogen assimilation involves the reduction of NO_3^- to nitrite (NO_2^-) by nitrate reductase (NR), followed by conversion of NO_2^- to ammonium (NH_4^+) in plastids by nitrite reductase (NiR) [1]. NH_4^+ is then incorporated into nitrogen compounds primarily through the GS/GOGAT cycle [2]. Nitric oxide (NO), in turn, is a reactive nitrogen species involved in signal transduction. Its metabolism is crucial for plant ontogenesis and adaptation to unfavorable environmental conditions [3]. NO can be synthesized enzymatically, for instance, via the reduction of NO_2^- by NR, and stored in a stable form as *S*-nitrosoglutathione (GSNO). The NO pool may be regulated, for example, through increased activity of GSNO reductase (GSNOR), which converts GSNO to oxidized glutathione (GSSG) and NH_4^+ [4]. Under stress conditions, overproduction of reactive nitrogen species (RNS) can lead to secondary nitrosative stress, resulting in cellular damage [5].

In barley exposed to five days of dual stress of salinity (50 or 100 mM NaCl) and infestation by *Aceria tosichella* (wheat curl mite, WCM), increased NR activity and NO fluorescence in tissues were observed. Under combined stress (50 mM NaCl+WCM), NO was synthesized in vacuoles at low pH, likely supporting the response to osmotic stress. In contrast, NO accumulation in the epidermis under WCM infestation and combined 100 mM NaCl+WCM stress may result from effector release by the mite. Additionally, a high glutathione (GSH) to GSSG ratio was observed across all treatment groups, potentially favoring GSNO storage. Moreover, expression GSNOR-encoding genes increased under 100 mM NaCl and 100 mM NaCl+WCM treatments, suggesting a potential role in NO regulation.

In conclusion, barley modulates nitrogen metabolism in response to dual stress of salinity and WCM infestation, utilizing NO primarily as a signaling molecule.

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Session 6

Reactive nitrogen species in growth and development

Part I

Decoding the role of nitric oxide in pollen development

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Pollen development is a critical phase of the plant reproductive cycle, and its sensitivity to environmental stress directly affects fertility and crop productivity [1]. While nitric oxide (NO) is a versatile signaling molecule with established roles in seed dormancy and plant-pathogen interactions [2], its involvement in male gametophyte development remains insufficiently characterized [3]. Studies about pollen tube growth have shown that it is negatively affected by the flux of exogenous NO which is an important part of the fertilization process and might be a chemical signal the female reproductive uses to guide pollen tubes through the stigma system [4,5] on the other hand, the involvement of NO in anther and pollen grain development and maturation remains unexplored.

The transcription factors TGA9 and TGA10, members of the bZIP family, are indispensable for anther dehiscence and pollen viability as the double mutant *tga9tga10* produces non-dehiscent anthers and exhibit a complete loss of pollen germination [1]. Redox regulation of these factors has been attributed to the activity of ROXY1 and ROXY2, which modulate cysteine residues likely influencing their DNA-binding or transcriptional activity [6]. However, despite the potential chemical similarity between thiol reduction and NO-dependent *S*-nitrosylation, no studies have yet demonstrated direct NO-mediated control of these transcription factors.

Our research aims to explore the post-translational landscape of NO signaling in pollen development, with a specific focus on S-nitrosylation as a regulatory mechanism. Using NO homeostasis mutants, cell-free protein expression systems and then a biotin-switch assays, we are investigating whether endogenous NO can influence key regulators of pollen maturation and function. Preliminary data reveal that endogenous NO disruption alters germination rates, implying the existence of targeted NO-dependent regulatory mechanisms.

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Regulation by NO and ripening of genes involved in carotenoid biosynthesis in sweet pepper (*Capsicum annuum* L.) fruits

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Carotenoids (Car) are a group of isoprene-derived pigments involved in the photosynthesis, protect cells from oxidative damage and serve as precursors for abscisic acid and vitamin A. During pepper fruits ripening, the conversion of chloroplasts into chromoplasts and the accumulation of Car occur. This bulk of carotenoids is responsible for the color change from immature green to ripe red fruits, and is linked to an enhancement of their nutritional value due to their antioxidant properties.

Nitric oxide (NO) has been proven to delay maturation and carotenoid accumulation in sweet pepper fruits [1], although the target Car biosynthesis genes on which NO acts had not yet been identified. Therefore, the aim of this work is to investigate the differential expression of carotenoid biosynthesis genes at ripening in pepper fruits and whether NO can alter such expression.

By RNA-Seq analysis, fifteen Car biosynthesis genes were detected in the transcriptome of sweet pepper fruits, twelve of which were differentially expressed by ripening. On the other hand, in pepper fruits subjected to NO treatment seven enzyme-encoding genes involved in the Car biosynthesis were differentially expressed, either down- or up-regulated. Among these, the *CaPDS* (*phytoene desaturase*), *CaBCH1* (*β-carotene hydroxylase 1*), *CaLCY-B1* (*lycopene β-cyclase 1*), *CaCCS* (*capsanthin/capsorubin synthase*) and *CaNCED1* (*epoxycarotenoid dioxygenase 1*) were down-regulated by NO. Conversely, *CaLCY-E* (*lycopene ε-cyclase*) and *CaNCED2* (*epoxycarotenoid dioxygenase 2*) were up-regulated by NO.

These findings reinforce the idea that carotenoid biosynthesis in sweet pepper is a complex pathway in which numerous genes partake, and whose expression is regulated depending on the physiological and environmental conditions.

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Acknowledgements:
Supported by ERDF co-financed grants from the Ministry of Science and Innovation (PID2023-146153NB-C21 and CPP2021-008703), Spain.

Nitric oxide impacts the proteome in embryonic axes of artificially aged apple (*Malus domestica* Borkh.) seeds

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Seed ageing impacts biodiversity preservation, the forestry and agronomy. The loss of seed vigour is associated with overproduction of reactive oxygen species (ROS) and the depletion of reactive nitrogen species (RNS) level that is typical for apple (*Malus domestica* Borkh.) seeds. ROS and RNS (RONS) react with the cellular components, including proteins, often leading to irreversible modifications. The protein quality control system is responsible for degrading modified proteins, preventing them from aggregating and causing damage. Apple embryos, isolated from seeds subjected to elevated temperature and humidity for more than 14 days, exhibit signs of ageing. Exogenous nitric oxide (NO) mitigates the detrimental effects of seed ageing. The aim of this work was to study the effects of NO treatment on the profiles of proteins modified by ROS (carbonylated) and RNS (nitrated), in embryonic axes isolated from apple seeds artificially aged for 7, 14, and 21 days. Moreover, the activity of the 20S proteasome and the changes in the ubiquitinated proteins were measured. NO treatment improved aged embryo germination by limiting the occurrence of morphological abnormalities in developing seedlings. Using mass spectrometry techniques, we identified proteins belonging to late embryogenesis abundant (LEA), heat shock protein (HSP), and seed biotin-containing protein (SBP) families that undergo carbonylation. Moreover, we discovered that NO treatment modulates the nitrated protein profile, and most of these changed proteins were connected with energy metabolism. Additionally, treatment of aged embryos with NO improved the activity of the 20S proteasome, revealing its role in protein degradation.

Session 7

Reactive nitrogen species in growth and development

Part II

Seed treatment with nitric oxide-releasing nanoparticles as a sustainable strategy to improve soybean growth and development: From the laboratory to the field

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Chitosan-based nitric oxide (NO)-releasing nanoparticles (NPs) have potential as agricultural biostimulants due to their controlled release of NO, enhancing plant resistance to stress conditions, such as drought, and promoting plant growth and productivity. This study aimed to evaluate the effects of applying NO-releasing chitosan NPs to soybean (*Glycine max* (L.) Merrill) seeds at different concentrations under laboratory and field experimental conditions. Initially, in the laboratory, artificially aged soybean seeds were treated with NPs at concentrations of 1, 2, 4, 8, and 16 mM of *S*-nitroglutathione (GSNO), along with a water control and a non-treated control. Principal component analysis revealed clear separation among treatments, highlighting positive effects of NO-releasing NPs in preserving seed vigour, especially at intermediate doses (4 and 8 mM GSNO). Under field conditions, these benefits were confirmed, showing a significant increase in plant emergence from treated seeds compared to the controls, as well as in increase in root *S*-nitrosothiol content. Morphological assessments indicated marked increases in root and shoot lengths, again emphasizing intermediate concentrations as the most effective ones. Additionally, an increased number of active root nodules was observed, indicating enhanced symbiotic associations with nitrogen-fixing bacteria. The leaf area index of treated plants was also superior, reflecting in greater photosynthetic capacity and vegetative development. Finally, the grain productivity significantly increased in treatments with NO-releasing NPs. These results indicate that NO-releasing chitosan NPs represent a promising strategy for enhancing soybean growth, resistance and productivity under adverse environmental conditions.

Acknowledgements:
Supported by CAPES, CNPq, FAPESP, Fundação Araucária/SETI, and INCT NanoAgro.

Seed priming with nitric oxide-releasing nanoparticles as a strategy to enhance maize performance under field conditions

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Climate change exacerbates abiotic stresses such as drought and high temperatures, significantly affecting crop productivity. Maize (*Zea mays* L.), due to its low phenotypic plasticity, is particularly vulnerable during early developmental stages. Therefore, technologies that unlock the crop's full genetic potential from emergence are crucial. Nitric oxide (NO) is a key signaling molecule that regulates physiological processes including growth, gas exchange, and antioxidant defense. However, its instability limits practical field applications. To overcome this, seed priming with chitosan-based nitric oxide-releasing nanoparticles (NO-NPs) offers a promising strategy for gradual and sustained NO delivery, potentially enhancing stress resilience. This study aimed to evaluate the effects of different NO-NP concentrations applied via seed priming on the morphophysiological traits of maize seedlings under field conditions. The experiment was conducted in Londrina, Paraná, Brazil, using a randomized block design with five replications. Results showed a significant quadratic dose-response relationship, indicating a dose-dependent effect. Among the treatments, the 4 mM NO-NP dose was the most effective, enhancing emergence speed index, shoot and root growth, biomass accumulation, and leaf area index. Physiologically, this dose also increased stomatal conductance and chlorophyll content (a, b, and total), along with alterations in the chlorophyll a/b ratio. These changes suggest enhanced light capture and carbon assimilation efficiency, reflecting higher photosynthetic activity, which supported early dry matter accumulation and cell expansion. In conclusion, seed priming with NO-releasing chitosan nanoparticles is an effective strategy to enhance early growth and physiological performance of maize seedlings under field conditions.

A quorum-sensing mutant of *Pseudomonas aeruginosa* promotes plant growth *via* nitrate reduction and transport and nitric oxide accumulation in roots

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Plants and bacteria communicate through chemical signaling, which determines plant growth promotion or repression. *P. aeruginosa* Pao1 behaves as a plant pathogen. However, a mutant defective on the production of the main autoinducer *N*-(3-oxododecanoyl)-*L*-homoserine lactone, termed *ΔlasI,* promotes growth and development [1,2]. Global gene expression analysis revealed the induction of genes for nitrate uptake and assimilation in Arabidopsis seedlings co-cultivated with *Pseudomonas aeruginosa* WT (PAO1) or *ΔlasI* mutants. Both bacteria upregulated the dual-affinity nitrate transceptor CHL1/AtNRT1/ NPF6.3 and the nitrate reductases NIA1 and NIA2. *CHL1-GUS* was induced in Arabidopsis primary root tips after transfer onto *P. aeruginosa ΔlasI* streaks at low and high N availability, whereas this bacterium required high concentrations of nitrogen to potentiate root and shoot biomass production and to improve root branching. Arabidopsis *chl1-5* and *chl1-12* mutants and double mutants in NIA1 and NIA2 nitrate reductases showed compromised growth under low nitrogen availability and failed to mount an effective growth promotion and root branching response even at high NH_4NO_3. WT *P. aeruginosa* PAO1 and *P. aeruginosa ΔlasI* mutant promoted the accumulation of nitric oxide (NO) in roots of both the WT and *nia1nia2* double mutants, whereas NO donors SNP or SNAP did not improve growth or root branching in *nia1nia2* double mutants with or without bacterial cocultivation. We conclude that 1) inoculation of *Arabidopsis* roots with *P. aeruginosa* Pao1 drives gene expression for improved nitrogen acquisition, 2) nitrate is required for growth enhancement of *ΔlasI* bacterized seedlings, 3) nitric oxide may act as a second messenger in *Arabidopsis-P. aeruginosa* interaction. Overall, disruption of the *LasI* quorum-sensing system of a bacterial phyopathogen changes the relationship with plants from growth repressing to biostimulant through nitrate nutrition and NO signaling.

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Poster sessions abstracts

Session 1

Reactive nitrogen species and metabolism

(P1) Heterologous expression of an unusual NOS and its impact on nitrogen metabolism in cyanobacteria

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Cyanobacteria are photosynthetic organisms capable of adapting to nitrogen (N)-deficient environments, making them excellent models for studying N metabolism regulation in photosynthetic organisms such as higher plants. N deficiency triggers various responses, including reduced growth, pigment degradation (e.g., phycobiliproteins), glycogen accumulation, and remobilization of organic N reserves such as arginine (Arg) [1]. Nitric oxide synthases (NOS) are enzymes that catalyze the oxidation of L-arginine to nitric oxide (NO) and citrulline. In our lab, we have characterized the NOS from the cyanobacterium *Synechococcus* PCC 7335 (SyNOS) [2]. In vitro assays demonstrated that SyNOS can also act as a NO dioxygenase, oxidizing NO to nitrate through its globin domain present at the N-terminus [3]. Current results suggest that SyNOS could participate in primary N metabolism by catabolizing Arg into nitrate, which could be reassimilated. The objective of this work is to analyze the effect of heterologous expression of SyNOS in the model cyanobacterium *Synechococcus elongatus* PCC 7942, which does not encode NOS. To this aim a recombinant strain expressing SyNOS under the IPTG-inducible promoter Ptrc was generated. IPTG induction in the recombinant strain allowed detection of SyNOS mRNA and protein by Real-Time PCR and Western blot, respectively, while decrease of Arg content informed on the enzymatic activity of SyNOS in *S. elongatus*. Importantly, SyNOS expression enhanced *S. elongatus* growth, phycocyanin degradation and glycogen accumulation under N-deficient conditions. Additionally, we are currently assessing the phosphorylation status of the N regulatory protein PII. These results provide new insights into NOS roles in cyanobacterial nitrogen metabolism.

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(P2) Nitric oxide and flavohemeproteins in barley root tips

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Nitric oxide (NO) is a gaseous molecule with signaling properties participanting in many physiological processes. A growing body of evidence indicates the involvement and cooperation of multiple pathways in plant NO generation, depending on the developmental stage, tissue and organ type and on the environmental conditions. In the past decades, numerous potential non-enzymatic and enzymatic NO synthetic pathways, localized into different cell compartments, have been described in plants [1,2]. Moreover the level of NO in plants is dependent also on the rate of NO metabolism and scavenging, which is controlled by several different molecules and proteins. In mammalian cells, NO was found to be a substrate for multiple members of the peroxidase superfamily under physiological conditions [3].

Using a pharmaceutical approach, we analyzed NO accumulation, emission and catabolism in barley root tips. The NO consumption by barley root cells was inhibited when flavohemeprotein inhibitors, such as azide, cyanide, diphenyleneiodonium and dicumarol (an inhibitor of the plasma membrane electron transport chain) were applied, while we also observed an increase in NO levels as well as a stimulated NO emission from the root tip cells during the inhibitors application. These results suggest a considerable NO consumption activity of cells in the barley root tips, which probably, besides the NO production, is a key mechanism involved in the regulation of NO level in barley root tips.

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(P3) Copper-dependent nitro-oxidative stress modifies *Phytophthora infestans*'s offensive strategy towards potato

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Our study aimed to evaluate the effect of copper (Cu) – an essential heavy metal - on the redox status and (patho)biology of *Phytophthora infestans* (Mont.) de Bary, the causative agent of potato late blight. The experiments were conducted on Avirulent (Avr) and virulent (vr) *P. infestans* isolates in reference to the potato (*Solanum tuberosum* L.) cv. Bzura (carrying *R1* and *R2-like* genes). Pathogen isolates were growing *in vitro* in the presence of Cu ions at conc. of 5 and 10 mg/L.

To gain insight into Cu-dependent nitro-oxidative status, levels of reactive oxygen and nitrogen species (RNS) were quantified. Briefly, Cu induced the formation of nitric oxide, peroxynitrite and nitroxyl in a dose-dependent manner. Then, to verify whether and to what extent Cu-dependent changes in the pool of RNS affect nitro-oxidative modifications of nucleic acids and proteins, specific biomarkers were quantified. Analysis of DNA and RNA oxidative modifications revealed slight changes in RNA, with approx. 15% higher level of 8-hydroxyguanosine in Avr isolate after Cu exposure. The parallel formation of 8-nitroguanine was increased in RNA of Avr *P. infestans* and DNA of the vr isolate. The detection of 3-nitrotyrosine content in proteins revealed an approx. 50% decrease in this modification in vr *P. infestans* as a result of Cu 10 mg/L. In the case of oxidative protein modifications, both Cu doses in the Avr isolate resulted in an approx. 2-fold increase in carbonylated protein (PC) content. In vr isolate Cu 10 mg/L resulted in an approx. 60% higher level of PC. Finally, using specific molecular markers and leaf tests, it was evaluated whether the noted Cu-dependent nitro-oxidative changes modify the pathogen's ability to cause disease symptoms in potato. To conclude, Cu induces nitro-oxidative stress in Avr/vr *P. infestans* structures, as evidenced by modifications to biomolecules and an offensive strategy against potato.

Acknowledgement:
Funded by National Science Centre – proj. no. UMO-2021/41/N/NZ9/01525.

(P4) Effect of chitosan-encapsulated *S*-nitrosoglutathione (GSNO) nanodonor on endogenous nitric oxide (NO) metabolism in *Brassica napus* seedlings

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NO plays a key role in plant growth, development, and stress signaling. One of its natural donors, GSNO, can be nanoencapsulated to improve delivery. In our study, we compared the effects of bulk GSNO, GSNO-loaded chitosan nanocapsules (GSNO-CHT), and empty chitosan (CHT) capsules on *Brassica napus* L. cv. GK Gabriella after a short root treatment. The encapsulated form released NO more effectively and slightly more steadily than the bulk compound. Interestingly, CHT itself influenced root cell wall components, like pectin, lignin, and callose, possibly making the tissue more rigid. Both GSNO-CHT and CHT also caused rhizosphere acidification, likely due to increased organic acid release. Using fluorescent labeling, we found that GSNO-CHT did not enter root cells during the 2-hour exposure, while empty CHT capsules interacted only with the outer cell layers. Importantly, GSNO-CHT significantly boosted *in planta* NO and *S*-nitrosothiol (SNO) levels without inducing nitrosative stress, unlike bulk GSNO, which triggered peroxynitrite formation and protein nitration. GSNO-CHT also avoided activating GSNO reductase, potentially allowing stronger NO/SNO signaling. Although several NO-related genes (*BnNIA1, BnGLB1, BnGLB2*) were upregulated, their expression didn't directly track with NO levels, suggesting broader or alternative roles. Altogether, our results highlight GSNO-CHT as a safe and effective NO delivery tool in plants, enhancing signaling while minimizing stress.

Acknowledgements:
This work was supported by the National Research, Development and Innovation Office of Hungary under grant No. K 135303 (Zs.K.) and prepared with the professional support of the University Research Scholarship Program Of The Ministry Of Culture and Innovation, Financed from the National Research, Development and Innovation Fund (EKÖP-24-3-SZTE-521).

(P5) Nitric oxide – polyamine cycle - hydrogen peroxide as the signaling triad regulating barley senescence

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Leaf senescence is a highly-controlled sequence of events comprising the final stage of development. Understanding its molecular mechanism is important for improving of crop yield and postharvest storage. In barley, dark-induced leaf senescence (DILS) leads to a more oxidative cellular environment, which lowers the production of nitric oxide (NO) and nitroxyl (HNO) while increasing hydrogen peroxide (H_2O_2) levels. Moreover, plant cells sense and respond to redox fluctuations through dynamic shifts in the levels of cellular polyamines (PAs).

Considering the obtained results of measurements of the levels of NO, HNO, H_2O_2, the Fv/Fm parameter, and the levels of PA in control conditions vs. conditions modified with PA metabolism inhibitors and H_2O_2 scavengers, it can be concluded that induced senescence disturbs the homeostasis of polyamines, which in turn contributes to senescence-dependent changes in the NO and H_2O_2 levels and vice versa.

Our findings suggest that NO production, facilitated by putrescine catabolism, plays a regulatory role in leaf senescence. We propose a functional connection between the PA cycle and key signaling molecules such as NO, HNO, and H_2O_2. This interconnected network — NO↔PA↔ H_2O_2 — may influence metabolic reprogramming, steering the plant toward either growth or senescence. A shift in redox balance that favours the reduction in levels of NO and HNO appears to be a critical factor in coordinating senescence in relation to PA metabolism. Metabolic reprogramming modulating this process by maintaining NO emission or adjusting polyamine levels to promote a more reductive cellular environment may confer anti-senescence effects.

Acknowledgements:
This work was supported by the National Science Centre, Poland (project numbers 2017/27/B/NZ9/02135 to EPL and 2018/29/B/NZ9/00734 to ESN).

(P6) Protein-protein interactions of GSNOR in *Arabidopsis thaliana* reveal potential regulators of nitric oxide homeostasis

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Nitric oxide (NO) is a conserved signaling molecule critical for plant growth and stress responses. In *Arabidopsis thaliana*, NO is stored as *S*-nitrosoglutathione (GSNO) and regulated by *S*-nitrosoglutathione reductase (GSNOR), which irreversibly reduces GSNO using NADH. GSNOR-null mutants (*hot5-2*) display developmental defects and reduced stress tolerance, highlighting GSNOR's role in NO homeostasis. To identify proteins interacting with GSNOR in *Arabidopsis thaliana*, we employed enzyme-catalyzed proximity labeling using a GSNOR-TurboID fusion transgenic line. The fusion protein successfully rescued the phenotype of *hot5-2*, indicating it is functional and does not disrupt plant growth. Lines expressing TurboID-YFP targeted to the nucleus or cytosol were used as controls. Mass spectrometry analysis identified several potential interactors, including superoxide dismutase 1 and non-symbiotic hemoglobin - proteins previously reported to be involved in NO homeostasis - as well as proteins associated with signaling and plant hormone regulation. Current work focuses on validating selected candidate interactors through bimolecular fluorescence complementation (BiFC) in *Nicotiana benthamiana* and biophysical assays to obtain quantitative interaction data. Altogether, these results will identify candidate interactors of GSNOR and offer new insights into the protein interaction network that regulates NO homeostasis in plants.

Acknowledgment:
This research was funded by the NSF, grant number MCB-181798.

Session 2

Reactive nitrogen species in abiotic stress

(P7) Impact of seed priming with plasma-activated water on physiologycal parameters of *Arabidopsis thaliana* L. seedlings

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In the context of contemporary global climate change dynamics, flora is increasingly exposed to substantial environmental stressors. Among these, drought constitutes one of the foremost abiotic challenges, significantly limiting crop productivity on a worldwide scale. Several strategies have been devised to mitigate drought-induced stress, with seed priming being recognized as a significant technique. This technique represents a cost-efficient and sustainable strategy with the capacity to enhance drought resilience and consequently improve agricultural productivity.

Plasma-based technologies enable the generation of priming agents that are both economically viable and efficient, while also minimizing environmental impact. Plasma-activated water (PAW) is produced utilizing cold plasma, which increases the concentration of reactive oxygen and nitrogen species (RONS) within the treatment medium. The concentration of RONS can be stabilized by the addition of zinc (Zn) into the solution [1], potentially enhancing the efficacy of PAW as a priming agent. Seeds of the wild type *Arabidopsis thaliana* L. (Col-0) were incubated in various treatment solutions [distilled water (HP), PAW, PA(W+Zn), PA(W+ZnO nanoparticle)] for a duration of 24 hours, under dark conditions, at 24 °C.

Drought conditions were simulated by administering a treatment of polyethylene glycol 8000 over a duration of three days, after an initial four-day cultivation period on agar media devoid of stress. Quantitative measurements pertaining to seedling growth were obtained, including parameters such as root length, hypocotyl length, cotyledon area, and stomatal density. Furthermore, assessments were conducted on cell viability, *in planta* zinc concentration, and the quantification of reactive oxygen and nitrogen species (RONS) within the plant, which encompass nitric oxide, peroxynitrite, superoxide radical, and hydrogen peroxide.

Reference:
[1] Kutasi K *et. al*. (2023) *Plasma Process Polym.* 20(3):2200143.

Acknowledgement:
The work was supported by the „Lendület" MOMENTUM project of the Hungarian Academy of Sciences (LP2023-14/2023).

(P8) Seed pre-treatment with plasma-activated water influences plant development, reactive oxygen and nitrogen species, and photosynthetic performance under osmotic stress

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Nowadays, drought has emerged as a significant issue. Innovative strategies are required to mitigate drought stress, thereby promoting healthier plant development and enhancing yields. The application of plasma-activated fluids offers a novel, environmentally friendly, and sustainable methodology. Seed pre-treatment serves as a technique which improves the growth and resilience of plants, resulting in enhanced germination, increased yield, and improved stress response.

The objective of our model experiments is to examine the impact of plasma-activated water (PAW) seed pre-treatment on osmotic stress tolerance. The proportions of reactive oxygen and nitrogen species (RONS) in PAW were altered through the incorporation of zinc ions (Zn). The following treatments were used for pea (*Pisum sativum* L. cv. Petit Provencal) seeds: distilled water (HP), PAW, PA(W+Zn) and Zn.

Following a 24-hour seed treatment period, the plants were cultivated for 10 days, after which they were subjected to a 72-hour osmotic stress treatment with 20% w/v polyethylene glycol (PEG8000). The use of PEG8000 significantly reduced stem- and primary root length as well as the number of lateral roots, which seed pre-treatments were unable to significantly ameliorate. Contrarily, the application of PAW seed pre-treatment effectively mitigated viability loss and resulted in bigger leaf area. Additionally, the PA(W+Zn) treatment resulted in longer lateral roots under stress induced by PEG8000. There was a notable increase in carotenoid and total chlorophyll content due to osmotic stress, though seed pre-treatments did not show any significant changes between treatments under stress conditions. Due to the enhancement of leaf development by PAW seed treatments, photosynthetic parameters were examined utilizing a porometer and OJIP (with FluorPen).

Acknowledgement:

The work was supported by the „Lendület" MOMENTUM project of the Hungarian Academy of Sciences (LP2023-14/2023).

(P9) Nitroxyl as a new regulator of hypoxia response in Arabidopsis

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Although nitric oxide (NO) is well recognized as a signaling molecule engaged in plant development and stress responses, the functional role of its single-electron reduced and protonated homolog-nitroxyl (HNO) is very limited. The triatomic molecule possesses unique chemical properties that facilitate its migration within a cellular environment, potentially serving as a signaling function. Our previous study demonstrated that HNO is endogenously formed in *Arabidopsis* cells, and hypoxia can promote this molecule's non-enzymatic formation. However, the functional implication of HNO in the plant responses to hypoxia stress remains unknown.

To get insight into how HNO regulates *Arabidopsis* response to hypoxia, we first used real-time electrochemical detection to quantify HNO in leaves during successive hours of stress and the subsequent recovery phase. The results showed that hypoxia provoked a *ca*. 25% increase in HNO formation, while the level dropped sharply in the recovery phase. Next, the spatiotemporal and tissue-specific effects of HNO and other NO redox forms on the expression of the hypoxia-responsive promoter elements (HRPE), were determined. HRPE-GUS reporter transgenic *Arabidopsis* line pretreated with the selected modulators showed that the presence of HNO reduced HRPE-GUS activity in roots exposed to hypoxia in comparison to plants treated with other NO redox forms, suggesting that HNO might act as a negative regulator of HRPE expression under low oxygen conditions. Finally, to clarify the role of HNO in hypoxia stress tolerance, seedlings of wild-type *Arabidopsis* were pretreated with the selected HNO modulators, followed by exposure to stress conditions. We found that scavenging of endogenous HNO significantly reduced the survival ratio of root tips under hypoxia.

Our findings indicated a complex NO/HNO signaling interplay in *Arabidopsis* under low oxygen conditions. HNO plays a regulatory role in hypoxia-related gene expression, favoring plant survival.

Acknowledgement:
This research was funded by National Science Centre – project no. UMO-2017/26/E/NZ4/00226.

(P10) NO/HNO balance and its anti-senescence potential in premature barley leaf senescence

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Exposure to stress factors might induce senescence, like dark-induced leaf senescence (DILS) [1], which was shown to trigger a significant redox balance shift favouring a decrease in production of signaling molecules such as nitric oxide (•NO) and newly discovered nitroxyl (HNO) in *Arabidopsis* [2]. The balance between them depends on the redox status of the cell [3]. In this study, we investigated the anti-senescence potential of •NO and HNO in barley seedlings during DILS.

7-days-old barley leaves were exposed to DILS for 10 days and treated with S-Nitrosoglutathione (GSNO), a •NO donor, or low oxygen conditions (1%, LOC), which ensures a reductive environment. In order to follow the progress of DILS after treatments, senescence markers were observed: expression of senescence marker genes (AGXT, ICL) was measured using qPCR [4], leaves were phenotyped at days 0, 3, 7 and 10 using Photon Systems Instruments, with special attention on maximum quantum yield of PSII parameter, and plant's redox status was determined by measurements of total antioxidant capacity and hydrogen peroxide (H_2O_2) content. •NO and HNO levels were established using electrochemical methods.

Premature senescence changes the cell's redox balance and promotes the accumulation of H_2O_2 and loss of both •NO and HNO. Senescence symptoms were reversed by both GSNO and LOC, with LOC and its increased HNO levels being more effective.

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Acknowledgements:
The work was supported by the National Science Centre Poland (2018/29/B/NZ9/00734 to E.S-N) and the Initiative of Excellence – Research University (076/34/UAM/0086 to W.K).

(P11) AsPgb1-mediated NO scavenging is associated with ethylene reduction and delayed drought-induced senescence in oat

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Nitric oxide (NO) has emerged as a key signaling molecule in abiotic stress responses, with its precise regulation essential to prevent drought-induced damage. Phytoglobins, which scavenge NO, play a critical role in modulating stress responses and maintaining cellular homeostasis under water deficit. This study explores the relationship between oat phytoglobin (*AsPgb*1) expression, in vivo NO and ethylene dynamics, and drought tolerance in *Avena sativa*, via comparative analysis of drought-sensitive (Flega) and drought-tolerant (Patones) cultivars. Real-time NO measurements using laser-based photoacoustic spectroscopy revealed elevated NO accumulation in Flega during moderate to severe drought, coinciding with increased oxidative stress and tissue damage. In contrast, Patones showed strong drought-induced *AsPgb*1 expression and reduced NO levels, suggesting a protective regulatory role of phytoglobins. In Flega, drought-induced NO also triggered upregulation of arginine decarboxylase (ADC) and increased putrescine biosynthesis, while Patones maintained lower ADC expression and polyamine accumulation. Additionally, higher ethylene production in Flega was linked to enhanced senescence, likely exacerbated by excess NO. Analysis of other oat genotypes supported a consistent inverse relationship between phytoglobin expression and drought susceptibility. Overall, the results highlight the importance of phytoglobins in NO homeostasis during drought and their potential as targets for breeding drought-resilient oat cultivars.

Acknowledgements:
This work was supported by the grant [PID2022-142574OB-I00] funded by MICIU/AEI /10.13039/501100011033, by FEDER, UE, and by Junta de Andalucia, Qualifica Project [QUAL21_023 IAS]. GMB is holder of RyC grant from the State Research Agency RYC2022-037656-I.

(P12) Nitric oxide-releasing nanoparticles as a tool for improving cotton drought tolerance

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The search for techniques to increase crop drought tolerance has intensified due to the rising frequency and severity of drought events driven by climate change. Nitric oxide (NO) stands out as a key signalling molecule in plants, playing a crucial role in activating responses to abiotic stresses. The objective of this study was to evaluate the effects of treatment with chitosan nanoparticles containing *S*-nitrosoglutathione (NPGSNO) on mitigating the water deficit impacts in cotton (*Gossypium hirsutum* L.). Experiment was carried out in September/2024 in a greenhouse at State University of Londrina, Londrina, Paraná, Brazil. We used completely randomized blocks, with 7 replicates and treatments consisted in doses of NPGSNO (0, 50, 100, 200, 400 e 800 µM). Cotton seeds cultivar TMG 22 GLTP were sown in pots containing clay soil from native landscape. When plants achieved V2 stage, the treatment was realized through the application of suspension containing the nanoparticles (90 mL) directly to the soil. After application, irrigation was suspended to induce water deficit. The stomatal conductance (g_s) was measured 24, 48, 72 and 96 h after treatment and wilting status was evaluated based on a visual scale. The relative water content (RWC) was also determined. g_s and RWC data was analysed through ANOVA, preceded by homogeneity of variances (Bartllet) and normality of residues (Shapiro-Wilk) tests. When significant, regression analysis was applied ($p < 0.01$). The wilting scores was compared using non-parametric test (Friedman test). Differences in g_s was founded only 96 h after treatment, with 400 µM leading to the highest values. Although no differences were found regarding RWC, 400 µM promoted the lowest wilting scores. This indicates a protective effect of NPGSNO, but further studies are necessary to unravel the mechanisms of action of these nanoparticles and consolidate this technique as management practice for cotton.

Acknowledgements:
Supported by CNPq, CAPES and INCT NanoAgro.

(P13) Effect of formaldehyde on *Chlorophytum comosum* and nitric oxide synthesis in plant cells

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Formaldehyde (FA), a prevalent volatile organic compound in indoor air, has been identified as a significant stress factor for plants. ***Chlorophytum comosum*** (Spider plant) is recognized for its notable phyto-purification potential; however, the molecular and physiological mechanisms responsible for its resistance to formaldehyde remain to be fully elucidated. Nitric oxide (NO) is a signaling molecule that plays a critical role in the regulation of the plant response to environmental stress. This regulation occurs through the influence of NO, e.g. on redox metabolism, gene expression, and stomatal apparatus function.

The objective of this study was to assess the role of NO in the response of ***C. comosum*** to formaldehyde exposure. To this end, a series of biochemical and confocal microscopic analyses were conducted. Levels of NO were determined using fluorescent probe (DAF-2DA). The number, morphology, and degree of opening of the stomata were also analyzed. The activity of nitrate reductase (NR), an enzyme involved in NO biosynthesis, was measured spectrophotometrically.

The results demonstrated an increase in NO levels in leaf tissues following FA exposure. This was accompanied by enhanced fluorescence, particularly in the cuticle and epidermis. Stomata showed increased density and a tendency to close. Furthermore, nitrate reductase (NR) activity increased, indicating activation of the NO biosynthetic pathway as a defensive mechanism.

The results of the study suggest that NO plays a multifaceted role in the ***C. comosum*** plants response to FA stress. Its involvement is observed at the molecular level (enzymatic regulation), cellular level (reactivity and fluorescence), and anatomical level (modification of stomata). The results of this study lend support to the hypothesis that NO may function as a defensive signaling molecule, thereby facilitating plant adaptation to the presence of toxic volatile organic compounds (VOCs) in the environment including FA.

(P14) Biological function of nitric oxide (NO) in stress response of transgenic flax (*Linum usitatissimum* L.) plants

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The function of nitric oxide (NO) in plant physiology and metabolism refers to the regulation of plant growth and development, as well as responses to abiotic and biotic stresses. The subjects of the present study were transgenic flax (*Linum usitatissimum* L.) plants with overexpression of medium-chain-length polyhydroxyalkanoates (mcl-PHA) synthase gene from *Pseudomonas aueruginosa*. It should be pointed out that these polymers are synthesized in bacteria in stress conditions. Former analyses indicated higher resistance of generated transgenic flax to pathogens and improved tolerance to salinity in comparison to non-transformed flax plants [1]. NO application has been shown to mitigate salt stress in several plant species. Thus, it was interesting to analyse NO emission in transgenic flax. The present study demonstrates that in fact the most resistant transgenic flax line (numbered as 11) emitted the highest level of NO under tested stress conditions (100 mM NaCl and 100 mM mannitol). The level of NO was accompanied with increased antioxidant capacity of extracts isolated from transgenic flax (line #11). The observed results proved the significance of the introduced gene in improvement of plant resistance to stresses and pointed to the role of reactive nitrogen species (RNS) in plant stresses.

Reference:
[1] Wróbel-Kwiatkowska M *et al.* (2022) *Plant Cell Tiss Organ Cult*. 151:123–132.

Session 3

Reactive nitrogen species in biotic stress

(P15) Spatio-temporal changes in the generation of nitric oxide during the interaction of tomato plants with the fungal pathogen *B. cinerea*

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The results of the preliminary studies suggest that *Trichoderma virens* TRS 106, obtained from the bank collection of the Department of Microbiology and Rhizosphere, Institute of Horticulture – National Research Institute, plays an important role as a biocontrol agent (BCA). It significantly increases the defence responses of tomato plants (*Solanum lycopersicum* L.) against *Botrytis cinerea*, slowing down the development of grey mold disease, accompanied by the biochemical changes in plants and their chloroplasts, including nitric oxide (NO) accumulation. The study revealed a two-peak increase in NO accumulation in plants pretreated with *Trichoderma* spores added to the soil 0–72 hours after *B. cinerea* inoculation. Temporary NO accumulation was also evident in chloroplasts. Analysis of chloroplast function based on photosynthetic pigment content, ultrastructure, and photosynthetic parameters (including the OJIP test) showed that strain TRS 106 reduced the negative effects of *B. cinerea* on chlorophyll a content, the functioning of the PSII photosystem, and structural changes to chloroplasts caused by *B. cinerea*. The results obtained allow us to conclude that the applied strain has a positive effect on the photosynthetic system and the state of the chloroplasts, which may be an important factor in protecting tomato plants against grey mold. As NO accumulation is observed in protected chloroplasts, future research will focus on evaluating the role of this molecule in *Trichoderma*-induced protection of chloroplasts and the interaction pathways of NO with other molecules involved in defence responses.

Acknowledgement:
This work was supported by the National Science Centre, Poland, by the grant „Intense smell in organic farming - the endogenous nitric oxide and sulfur-containing volatile compounds in the protection of tomato plants against gray mold disease, based on the use of microbiological biocontrol agents” (Grant no. 2023/51/D/NZ9/01972).

(P16) GSNO treatment impacts on phytohormone levels in sweet cherry during storage

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Nitric oxide (NO) has positive effects on fruit quality during postharvest storage, but the mechanisms by which it exerts the effects are not fully understood (Buet et al., 2021). Here we studied the impact of *S*-nitrosoglutathione (GSNO), an NO donor, on phytohormone levels, quality and health of sweet cherry (*Prunus avium*) var. Bing, during postharvest storage. Fruits were immersed 5 min in 0.1 mM GSNO or distilled water (control); and stored at three conditions: 1) 7 d at 20 ± 2°C, 70% RH (Shelf life, SL); 2) 30 d at 0.0 ± 0.5°C, 95% RH (Cold Storage, CS); and 3) 30 d at 0.0 ± 0.5°C, 95% RH followed by 7 d at 20 ± 2°C, 70% RH (CS+SL). As compared to control, GSNO treatment reduced abscisic acid level in fruits after SL, while increased it under CS and CS+SL. Salicylic acid levels were reduced by 50% after CS and CS+SL in GSNO-treated cherries. Regarding gibberellins, GA_1 content was 2.4-fold higher, and GA_3 content was 2-fold lower in GSNO-treated fruits after SL; while both of them were decreased after CS. No changes in indole-3-acetic acid levels were observed regardless of the treatment and storage conditions. GSNO treatment consistently preserved fruit firmness without changes in skin color, titratable acidity, pH nor soluble solids content at all storage conditions. GSNO-treated fruits showed lower natural disease incidence under the three storage conditions. The associated pathogens were isolated and identified: *Alternaria* spp., *Cladosporium* spp. and *R. stolonifer*. *In vitro* assays ruled out a direct antifungal effect of GSNO against four common postharvest pathogens (*A. alternata*, *B. cinerea*, *C. herbarum* and *P. expansum*). These findings contribute to our understanding of the role of exogenous NO in maintaining postharvest sweet cherry quality and reducing disease incidence, likely through modulation of stress-related phytohormones and plant-pathogen interactions, which result promissory for the development of sustainable strategies for fruit conservation.

Reference:
Buet A *et al*. (2021) *J Plant Growth Regul*. 40(6):2286-2302.

(P17) Nitrate reductase-produced nitric oxide in Chinese cabbage leaves during the hypersensitive cell death against the non-adapted *Xanthomonas* bacteria

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Nitric oxide (NO) produced in plants is involved in diverse physiological changes during development and responses to external stimuli. Chinese cabbage showed specific increases in NO levels in the inoculated leaves against a non-adapted bacteria *Xanthomonas vesicatoria* (*Xv*), concomitantly with hypersensitive cell death (HCD) occurrence. Three NO-releasing compounds, sodium nitroprusside, *S*-nitrosoglutathione and 3-morpholinosydnonimine, differentially affected *in vitro* bacterial growth of an adapted bacteria *X. campestris* pv. *campestris* (*Xcc*) and non-adapted *Xv*. Gene expression and enzyme activity of nitrate reductase (NR) were also preferentially activated in the *Xv*-inoculated leaves. Highly increased arginine but decreased citrulline were found in the *Xv*-inoculated leaves. Pretreated tungstate, *L*-NAME and uric acid led to differential changes in fresh weight, electrolyte leakages, NO production and lipid peroxidation of *Xcc*- and *Xv*-inoculated leaves. The *Xv*-mediated NR activation was differently regulated by endogenous NO and peroxynitrite. Pretreated tungstate provided an unfavourable environment for *Xcc* bacterial growth *in planta*, but suppressed *Xv* growth in the leaves was alleviated by tungstate. Exogenous SNP decreased electrolyte leakages in the *Xcc*- and *Xv*-inoculated leaves, and increased *Xcc* bacterial proliferation in leaves. Augmented NR activity in the *Xv*-inoculated leaves was compromised by SNP application. These results suggest that endogenous and exogenous NO can regulate plant cell death and immunity differently in the Chinese cabbage leaves in response to an adapted *Xcc* and non-adapted *Xv* invasion.

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(P18) Nitric oxide defensive potential in Arabidopsis -Turnip mosaic virus (TuMV) interaction

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TuMV is one of the most economically important plant viruses worldwide. It has a very unique host range infecting over 43 families, which makes TuMV widely spread in natural environment [1]. This fact creates worldwide research pressure for investigation and development of new approaches to understand the complexity of interaction of TuMV with plant-host and in identification of crucial molecules and processes for resistant responses. The world main research focus in plant host - virus interaction is aimed for ROS as factor in signal transduction network and molecules which enables programmed cell death (PCD). However, many investigations not fully explore potential role of nitric oxide (NO) as an endogenous signaling molecule in plants during viral infection. Therefore, the aim of our study was multilevel TuMV infection course after inoculation of *A. thaliana cue1-5* and *cue1-6* mutants. Both mutants types revealed NO overproduction [2] in comparison to Col-0. Ultrastructural analysis of *cue1-5-, cue1-6-* TuMV interaction indicated lack of viral cytoplasmic inclusion typical for TuMV and virus particles were presented only in vacuoles. Furthermore, ultrastructural alterations were accompanied by significantly changed of classical markers of host-plants response to viral infection. Our findings indicated that *cue1-5* and *cue1-6* displayed significantly less TuMV accumulation (based on *TuMV-CP* gene validation) associated with dynamic induction of defensin (*AtPR-12*) gene expression between 7 and 21 dpi. Whereas, susceptible Col-0- TuMV systemic reaction, significantly induced *AtPR1* gene expression (between 3-21 dpi). It was correlated with increased TuMV level and reduction of *AtPR-12* gene. Above observations suggest that *A. thaliana* mutants characterized NO overproduction revealed more resistant reaction type than Col-0. These results may help provide a step forward in better understanding of the mechanisms of NO involvement in regulations of *A. thaliana*–TuMV interactions.

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(P19) Unraveling the role of *S*-nitrosylation during hypersensitive resistance to powdery mildew

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Nitric oxide (NO) is a key signalling molecule that regulates diverse physiological and immune responses in plants. One of its main mechanisms is *S*-nitrosylation, a reversible post-translational modification of cysteine residues that can alter protein function. This study investigates the role of *S*-nitrosylation during infection of barley (*Hordeum vulgare*) by the powdery mildew fungus *Blumeria graminis*. Prior research showed rapid NO accumulation in resistant barley epidermal cells undergoing a hypersensitive response (HR), suggesting NO involvement in early defense signaling. To identify NO-regulated targets, we inoculated leaves of the resistant barley genotype P01, which carries the *Mla*1 resistance gene, and isolated proteins from both epidermal cells and whole leaf tissue during HR development. *S*-nitrosylated proteins were labelled using the biotin-switch technique and enriched via streptavidin-agarose purification. Mass spectrometry revealed clear pathogen-induced changes in *S*-nitrosylation patterns in the epidermal fraction, while total extracts showed minor shifts, likely due to dilution by uninfected tissue. Identified *S*-nitrosylated proteins included several known enzymes involved in plant immunity. E.g. peroxidases catalysing hydrogen peroxide dependent reactions (one with >300-fold increase) and a PATATIN-like protein 5 related to lipid signaling (~100-fold increase). Enzymatic activity assays in healthy versus infected P01 plants supported the functional involvement of these proteins in defense responses. These findings highlight the role of *S*-nitrosylation in plant immunity and its importance in resistance to powdery mildew.

Acknowledgements:
This work was supported by grant [PID2022-142574OB-I00] funded by MICIU/AEI /10.13039/501100011033, by FEDER, UE, and Junta de Andalucia, Qualifica Project [QUAL21_023 IAS]. GMB is holder of RyC grant from the State Research Agency RYC2022-037656-I.

Session 4

Reactive nitrogen species in growth and development

(P20) Effect of *S*-nitrosoglutathione-containing chitosan nanoparticles on the development of *Cecropia pachystachya* seedlings under field conditions

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Nitric oxide (NO) is a signaling molecule that regulates various physiological processes in plants, such as growth and responses to abiotic stress. However, its direct application is limited due to its chemical instability. The nanoencapsulation of NO donors, such as *S*-nitrosoglutathione (GSNO), into chitosan nanoparticles (NPs) represents a promising strategy to ensure controlled and effective release of this bioactive compound. This study aimed to evaluate the effects of applying GSNO-loaded NPs on the development of *Cecropia pachystachya* seedlings under field conditions, in comparison with a commercial formulation (Arbolina) and an untreated control. The seedlings received three applications of the treatments on alternate days prior to planting, followed by a booster application approximately four months after transplantation. Throughout the experimental period, monthly evaluations of gas exchange parameters were conducted. After five months, the final assessment was carried out, and the leaf area of the plants was measured. The results showed that the GSNO-NP treatment significantly increased leaf area compared to the other treatments, standing out as the only group with statistically superior means. Regarding photosynthetic rate, the second evaluation after planting showed that GSNO-NPs outperformed the control and were similar to the commercial formulation. In the fourth evaluation, the GSNO-NP treatment maintained superiority over the commercial formulation and showed similar performance to the control. These findings highlight the potential of NO-releasing NPs as an effective tool to enhance early seedling growth in forest restoration projects, contributing to higher plant establishment success under field conditions.

Acknowledgements:
Supported by CAPES, CNPq, FAPESP, and BiodivERsA, under the BiodivClim ERA-Net COFUND program, with the funding agencies Araucária Foundation (Brazil, Paraná), ANR (France) e BMBF (Germany).

(P21) Effects of seed priming with *S*-nitrosoglutathione-loaded chitosan nanoparticles on the early development of Atlantic Forest tree seedlings

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Seed priming with nitric oxide (NO) donors is a promising, low-cost technique to improve germination, seedling vigor and stress tolerance during early plant development. This study evaluated the effects of seed priming using polymeric nanoparticles (NPs) loaded with *S*-nitrosoglutathione (GSNO) on three Atlantic Forest tree species: *Cecropia pachystachya* Trécul (Urticaceae), *Cedrela fissilis* Vell. (Meliaceae), and *Senna macranthera* (DC. ex Collad.) H.S.Irwin & Barneby (Fabaceae). Seeds were treated with chitosan-based NPs containing GSNO at concentrations of 0.1, 0.25, 0.5, 1, 2.5, and 5 mM, as well as hydropriming and non-primed controls. Seeds were immersed in the formulations for 10 minutes under constant stirring, then dried in the dark for 24 hours before sowing in sand-filled pots in a greenhouse. The measured parameters were emergence percentage, emergence speed index, main root, shoot and lateral root length, lateral root density, vigor index, root and shoot dry masses and root-to-shoot ratios for both length and dry mass. In *C. pachystachya*, the lateral root length increased by approximately 64.8% compared to hydropriming. In *C. fissilis* and *S. macranthera*, hydropriming led to imbibition damage, which was partially prevented by NPs GSNO. In *C. fissilis*, main root length was enhanced by NPs GSNO, with the highest value recorded at 2.5 mM. In *S. macranthera*, lateral root length increased by 27.1%, 27.6%, and 28.1% at 0.25, 0.5 and 1 mM, respectively. Shoot dry mass increased by 10.3% at 2.5 mM, while the root-to-shoot dry mass ratio was elevated by 32.4%, 27.0%, and 24.3% at 0.25, 0.5 and 1 mM, respectively, compared to hydropriming. These results highlight the potential of seed priming with NO-releasing NPs to enhance early seedling development in forest restoration efforts. However, the effectiveness of the technique varies among species, indicating the need for further studies and for adapting the approach to species-specific requirements.

Acknowledgements:
Supported by CAPES, CNPq, FAPESP, Fundação Araucária/SETI (NAPI Biodiversidade) and INCT NanoAgro.

(P22) Transcriptome rewiring evoked by the intracellular location of Phytoglobin 2 during the induction of Arabidopsis somatic embryogenesis

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Arabidopsis somatic embryogenesis (SE) is a suitable system to investigate cell plasticity. In the presence of auxin, somatic cells reprogram their developmental fate and embark in a new embryogenic path culminating to the formation of somatic embryos. This reprogramming is mediated by Phytoglobin 2 through its nitic oxide (NO) scavenging activity. Using a previously characterized dexamethasone (DEX) inducible system we were able to target or exclude Pgb2 from the nucleus. Nuclear Pgb2 retention had no effect on SE while its exclusion increased embryogenic competence by favoring formation of embryogenic tissue and somatic embryos. Transcriptome analyses revealed that exclusion of Pgb2 from the nucleus triggered early (3h) changes in gene expression centered around alterations of ubiquitination processes which were concomitant to a down-regulation of the transcription factor MYC2. Depression of genes implicated in amino acid synthesis and increased genes participating in auxin synthesis and response delineated the acquisition of embryogenic competence. A clear transcriptional signature observed during the first 12 of SE induction by the absence of Pgb2 from the nucleus was a shift in expression of stress-response (water and oxidative stress) related genes to genes regulating organ formation and meristem development. Collectively these studies demonstrate that by regulating NO homeostasis, changes in intracellular Pgb2 localization evoke major transcriptome rewiring linked to the somatic-embryogenic transition delineating the onset of SE induction.

(P23) Optimization of *S*-nitrosoglutathione (GSNO) treatment for genetic screening of *Arabidopsis thaliana*

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Nitric oxide (NO) is a key signaling molecule in plants; it regulates growth, development and stress responses. The identification of new NO-related genes is necessary for a deeper understanding of the regulation by NO. In our work, we use genetic screening to identify NO-hypersensitive or insensitive lines from the Conditional cDNA Overexpressing System (COS) collection containing 40,000 transgenic *Arabidopsis thaliana* lines. For this, it is necessary to optimize the conditions of the external NO donor treatment. In my work, I used *S*-nitrosoglutathione (GSNO) in concentrations of 100-1500 µM, and different treatment methods on wild-type *A. thaliana* (Col-0) plants grown on agar medium. As a control, we set up a light-inactivated GSNO treatment (8h, 1500 µmol m^{-2} s^{-1}). Of the applied GSNO concentrations, spraying with 1250 µM was ca. 60% shortening of the primary root length compared to the control, and this proved to be an effect of sufficient magnitude to allow us to perform the screening of a large number of lines later on. We also optimized the GSNO concentration for experiments on estradiol medium and in the meantime we started the COS library screen.

Acknowledgement:
The work was supported by the “Momentum” program of the Hungarian Academy of Sciences (LP2023-14/2023).

(P24) Variations in endogenous NO generation influence the peroxisomal and the redox metabolism in Arabidopsis leaves

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Nitric oxide (NO) is a gasotransmitter that exerts signaling functions in plants. Using 30-day-old *Arabidopsis thaliana* plants both wild type and transgenic lines with different NO content (*Atnoa1* and *Atnox1/cue1*), the biochemical analysis in leaves of key components of the metabolism of reactive oxygen species (ROS), NADPH, NO, and H_2S was carried out. The obtained results indicate that the imbalance of the endogenous cellular NO triggered differential changes in many of the analysed biochemical parameters including the protein profile due to post-translational modifications such as *S*-glutathionylation, *S*-nitrosation, and tyrosine and tryptophan nitration. It was remarkable the differences observed in the activity of the antioxidant enzyme catalase and the H_2O_2-generating glycolate oxidase, two key peroxisomal enzymes involved in the ROS metabolism of these organelles. The gene expression of the *polyamine oxidase 4* (*POD4*), which encodes the peroxisomal H_2O_2-generating POD4, was also altered as a consequence of the capacity of the endogenous NO production. Furthermore, the pattern of the H_2S-generating L-cysteine desulfhydrase (LCD) isoenzymatic activity was also affected. These data provide new biochemical evidence of how under physiological conditions, NO can affect the peroxisomal metabolism, a subcellular organelle with a very active nitro-oxidative, ROS, NADPH, and H_2S metabolism.

Acknowledgement:
Research supported by European Regional Development Fund co-financed grants from the Ministry of Science and Innovation (PID2023-146153NB-C21 and CPP2021-008703), Spain.

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