FONDAZIONE Ce.U.B.

Workshop on Plant Biology 2026

Centro Residenziale Universitario di Bertinoro
25-27 February 2026

S.I.B.V.

SOCIETA' ITALIANA di BIOLOGIA VEGETALE

Workshop SIBV 2026

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Conference venue

Centro Residenziale Universitario di Bertinoro (Forlì-Cesena)

Conference Language

The official conference language is English

Conference Dinner on February 25

Ca’ de Bè – Osteria Enoteca, Piazza della Libertà, 9/b

GENERAL INFORMATION

Wednesday, February 25, 2026

01:30 pm **Registration and Welcome buffet**

02:15 pm **Workshop Opening**
Francesco Loreto, President of the Italian Society of Plant Biology – *University of Napoli Federico II*

Session 1: Adaptation mechanisms to environmental stress

Chairs: Aldo Sutti, Ginevra Marie Eloise Peppi

Invited:

02:30 pm **Spatiotemporal Integration of Environmental Stimuli in Plants: Towards a Single-Cell Perspective**
Paolo Triozzi PhD – *University Sant'Anna of Pisa*

Oral Presentations:

03:10 pm **Evaluation of reduced-activity Plant Cysteine Oxidase 4 variants effects on submergence tolerance in *Arabidopsis thaliana***
Arianna Del Greco - *University of Pisa*

03:25 pm **Strigolactones-gibberellin crosstalk via the miR319-LANCEOLATE module, and its relevance to stomatal functioning in tomato**
Shuo Liang – *University of Torino*

Flash Talks:

03:40 pm **Role of thallium hyperaccumulation in plant protection against biotic stress: insights from *Silene latifolia* and *Botrytis cinerea*.**
Beatrice Chiavacci, *University of Firenze*

03:45 pm **Priming-induced DDR (DNA Damage Response) signatures contributing to heat stress tolerance in tomato (*Solanum lycopersicum* L.)**
Ridwan Diaguna - *University of Pavia*

03:50 pm **An Integrative Multi-Omic Approach to Decode Adaptive Strategies of *Atropa belladonna* in Saline Environments**
Rosa D'Alessandro - *University of Salerno*

03:55 pm **Investigating stress memory in tomato plants exposed to recurrent water deficit**
Giulia Giannetti - *IPSP-CNR, Torino*

04:00 pm **COFFEE BREAK**

Oral Presentations:

04:45 pm **Trophic transitions from dark to light in the unicellular red alga *Galdieria phlegrea***
Rosanna Bossa - *University of Napoli Federico II*

05:00 pm **Understanding the impact of photoprotection mechanisms on ROS regulation in the moss *Physcomitrium patens***
Francesco Paolo Tranne - *University of Padova*

05:15 pm **Secretion and Regulation of Pectin Methylesterases as Mechanisms of Plant Adaptation to Environmental Stress**
Gabriele Pecatelli – *University of Roma "La Sapienza"*

05:30 pm **Transcriptomic and metabolic reprogramming of durum wheat under polystyrene nanoplastics exposure**
Benedetta Pizziconi – *University of Roma Campus Biomedico*

05:45 pm **Activity organized by SIBV Junior members: Escape Plant Lab**

08:00 pm **GET TOGETHER PARTY DINNER AT CA' DE BÈ RESTAURANT**

Thursday, February 26, 2026

Session 2: Plant Nutrition and Metabolism

Chairs: Davide Canini, Shraddha Shridhar Gaonkar

Invited:

09:00 am **Phosphoribulokinase: how to torment an enzyme to study carbon metabolism**
Libero Gurrieri –*University of Bologna*

Oral Presentations:

09:40 am **Heterologous expression of phosphoenolpyruvate carboxylase in *Nannochloropsis oceanica***
Jacopo Gelmetti - *University of Verona*

09:55 am **Iodine: a new weapon in the battle against abiotic stress**
Angelo Candito – *University Sant'Anna of Pisa*

10:10 am **Investigation of the metabolic coordination in diazotrophic cyanobacteria using genetically encoded fluorescent probes.**
Filippo Fiorin - *University of Padova*

Flash Talks:

10: 25 am **Physiological and Molecular Effects of Exogenous Strigolactone-like Molecules in Nutrient Use Efficiency in Tomato**
Ruihan Shen – *University of Torino*

10:30 am **Re-Waste: a green, sustainable and circular strategy for crop resilience**
Lucrezia Anastasia Ortelli - *University of Tuscia*

10:35 am **Recombinant production and characterisation of chloroplastic pentose phosphate pathway dehydrogenases from *Chlamydomonas reinhardtii***
Noemi Russo - *University of Napoli Federico II*

10:40 am **Structural determinants of functional and regulatory features of photosynthetic phosphoglycerate kinase**
Tancredi Bin - *University of Bologna*

10:45 am **COFFEE BREAK**

11:20 am **Activity organized by SIBV Junior members: Project Design**

13:00 pm **LUNCH**

Session 3: Development and signal transduction

Chairs: Emma Olmi, Margherita Gagliano

Invited:

02:30 pm **Keeping Hormones in Check: Homeostatic Control of Auxin and Jasmonate Action**
Federica Brunoni –*University of Verona*

Oral Presentations:

03:10 pm **Isolation of extracellular vesicles and characterization of their role as shuttle of biologically active small RNAs in intercellular communication**
Tommaso Sanson - *University of Verona*

03:25 pm **The role of HPCA1 and GLR3.3 in ROS perception and long-distance signal propagation after wounding in *A. thaliana* seedlings**
Adriana Furlani - *University "Roma Tre"*

03:40 pm **Dissecting Oligogalacturonide-Mediated Pathogen Resistance and Root Development**
Sara Di Renzo - *University of Roma "La Sapienza"*

Flash Talks:

03:55 pm **NADKinases-Calmodulin-dependent as driver for pollen tube tip growth**
Fabio Palmigiani - *University of Milano*

04:00 pm **Dissecting the Mitochondrial Unfolded Protein Response (UPRmt): From Stress Signaling to Physiological Acclimation in *Arabidopsis***
Anna Maria Placentino, *University of Padova*

04:05 pm **Balancing Growth and Defense: Role of TOR and SnRK1 Kinases in Biotic Stress and Cell Wall Damage Responses in *Arabidopsis***
Antea Mariani, *University of Roma "La Sapienza"*

04:10 pm **Seed morphophysiological heterogeneity in Lathyrus sativus accessions**
Davide Pezzini - *University of Pavia*

04:15 pm **COFFEE BREAK**

04: 50 pm **Activity organized by SIBV Junior members: Project presentation and evaluation**

08:00 pm **DINNER**

Friday, February 27, 2026

Session 4: Emerging technologies

Chairs: Nicholas Rizzetto, Amedeo Moine

Invited:

09:00 am **Spectral signatures potentially allow real-time, nondestructive identification of root species in the field**
Marco Lombardi, *Aarhus University*

Oral Presentations:

09:40 am **dsRNA production in cyanobacteria and microalgae for biocontrol of invasive species *Popillia japonica***
Elia Battagini, *University of Verona*

09:55 am **Assessing an In Vivo Approach to Validate CRISPR-Cas9 RNA Guides in Durum Wheat**
Francesca Orlando, *University of Tuscia*

10:10 am **From *Arabidopsis* to crop-relative species: unrevealing the hidden world of long-distance Ca^{2+} signalling in plants**
Bianca Maria Orlando Marchesano, *University of Milano*

Flash Talks:

10:25 am **IoT Microclimate Monitoring: Single-Plant Microchambers for Precision Phenotyping**
Giulia Fiorini, *University of Napoli Federico II / IPSP-CNR*

10:30 am **Cold plasma priming as a strategy to burst rice tolerance to water scarcity**
Teodora Chiara Tonto, *University of Roma Campus Bio-Medico*

10:35 am **Mechanisms of plant stress adaptation and physiological regulation induced by olive by-product-derived elicitors**
Giulia Caminada, *University of Roma "La Sapienza"*

10:40 am **Evaluation of wood distillate and Spirulina biostimulants to increase nickel hyperaccumulator-based phytomining in serpentine soil**
Pablo Carril Vaglini, *University of Firenze*

10:45 am COFFEE BREAK

Oral Presentations:

11:10 am **Expansion of the plant CRISPR-Cas platform: evaluation of the genome editing efficiency of the CoCas9 nuclease in *Solanum lycopersicum* hairy roots**
Marco Renzetti, *University of Roma "La Sapienza"*

11:25 am **Plasma priming as a tool for modulating germination, chemical and transcriptional profiling in *Cannabis sativa* seeds**
Nicola Bosco, *University of Pavia*

11:40 am **Seed biopriming in frames: understanding and improving its effectiveness through image-based analyses**
Conrado N. Dueñas, Jr, *University of Pavia*

11:45 am **CONCLUDING REMARKS**
Francesco Loreto, President of the Italian Society of Plant Biology – *University of Napoli Federico II*

01:00 am LUNCH

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Oral Presentations

Session 1: Adaptation mechanisms to environmental stress

Spatiotemporal Integration of Environmental Stimuli in Plants: Towards a Single-Cell Perspective

Paolo M. Triozzi *(1), Luca Brunello (1), Giacomo Novi (1), Gianmarco Ferri (2), Francesco Cardarelli (3), Elena Loreti (4), Mariano Perale (5), Pierdomenico Perata (1)*

(1) PlantLab, Institute of Plant Sciences, Sant'Anna School of Advanced Studies, 56010 Pisa, Italy
(2) Fondazione Pisana per la Scienza, 56017 Pisa, Italy
(3) Laboratorio NEST, Scuola Normale Superiore, Piazza S. Silvestro, 12, 56127 Pisa, Italy
(4) Institute of Agricultural Biology and Biotechnology, National Research Council, 56124 Pisa, Italy
(5) Centro de Biotecnología y Genómica de Plantas, Universidad Politécnica de Madrid (UPM), Campus de Montegancedo UPM, Pozuelo de Alarcón, 28223 Madrid, Spain

Plants constantly integrate environmental stimuli across different tissues and times of the day to optimize growth and development. During this continuous adjustment, oxygen availability represents a highly dynamic stimulus that is frequently limited by both external environmental stresses and internal factors. While acute hypoxia following flooding and chronic hypoxia in plant meristems have been extensively described, the existence of spatiotemporal oxygen dynamics under fluctuating environmental conditions has remained largely unexplored in plants.
In this study, we demonstrate that young, emerging *Arabidopsis* leaves undergo distinct spatiotemporal changes in internal oxygen levels. We reveal that at night, when photosynthesis ceases, a high respiration rate triggers a transient drop in endogenous oxygen levels within these developing organs. Furthermore, we show that the adaptation to this phenomenon, termed cyclic hypoxia, depends on the activity of ETHYLENE RESPONSE FACTORS type VII (ERFVIIs), which are the main transducers of hypoxia responses in land plants. Through ERFVII activity, cyclic hypoxia acts as a metabolic clock, coordinating leaf growth with endogenous oxygen and carbon availability. With the rapid development of single-cell technologies, we aim to decipher the transcriptomes of individual cells experiencing cyclic hypoxia in emerging leaves, paving the way for a high-spatiotemporal-resolution map of oxygen signaling during leaf development.

Evaluation of reduced-activity Plant Cysteine Oxidase 4 variants effects on submergence tolerance in *Arabidopsis thaliana*

Arianna Del Greco *(1), Anna Dirr (2), Mikel Lavilla Puerta (2), Emily Flashman (2), Beatrice Giuntoli (1)*

(1) University of Pisa
(2) University of Oxford

Plants have evolved a wide range of adaptations to face environmental stresses, as their sessile nature exposes them to a wide range of environmental changes. The increasing frequency of extreme environmental stresses associated with climate change, such as flooding, has intensified the interest in understanding and improving plant low-oxygen responses. A central component of plant hypoxic response is Group VII Ethylene Response Factors (ERF-VIIs) transcription factors, which regulate the expression of genes involved in adaptation to hypoxia. In normoxic conditions, Plant Cysteine Oxidases (PCOs) catalyse the oxidation of ERF-VIIs. This oxygen-dependent step of the Cys/Arg N-degron pathway leads to the degradation of the ERF-VIIs, preventing the activation of hypoxia-responsive genes. Under hypoxic conditions, reduced oxygen availability lowers PCO activity, resulting in the stabilization of ERF-VIIs which can initiate the expression of genes involved in the hypoxic response. For this reason, modulating ERF-VII abundance through PCO activity manipulation represents a promising strategy to enhance submergence tolerance in plants. One potential approach to enhance submergence tolerance is to reduce PCO activity, thereby modulating ERF-VII basal levels, accelerating their accumulation, or prolonging their stability under low-oxygen conditions. Using a combined structural and functional analysis of active-site residues, together with comparison to related enzymes, PCO4 variants were generated and subjected to biochemical and kinetic characterization, from which two variants emerged as particularly promising. The two variants were tested in vivo by expressing them in Arabidopsis thaliana, and their survival and recovery rates were compared with those of wild-type plants. Both variants showed significantly improved survival following submergence, supporting the conclusion that engineering PCO4 activity is a viable strategy to improve flood tolerance in plants.

Strigolactones-gibberellin crosstalk via the miR319-LANCEOLATE module, and its relevance to stomatal functioning in tomato

***Shuo Liang**, Nihan Sahin, Andrea Schubert, Fabio Tebaldi Silveira Nogueira, Francesca Cardinale*

University of Turin, Dipartimento di Scienze Agrarie, Forestali e Alimentari – DISAFA

Plant stomatal regulation is essential for balancing water conservation and gas exchange. Strigolactones (SLs) are carotenoid-derived phytohormones involved in abiotic stress tolerance and plant development. The conserved role of SLs in modulating stomatal movement has been reported. miR319 is a conserved miRNA family which mainly negatively regulates TCP transcription factors, such as LANCEOLATE (LA). LA interacts with PROCERA (PRO), the only DELLA protein in tomato, a repressor of gibberellin (GA) signaling. Interestingly, PRO can promote stomatal closure in tomato in an ABA-dependent manner. Since SLs can control the miR319-LA module, and modulate GA content, our research aims to investigate whether miR319-LA and GAs mediate the SL signal in stomatal regulation in tomato. Leaf spray treatment experiments were conducted using the synthetic SL analogue GR245DS on LA and DELLA mutant lines in both M82 and MT backgrounds, followed by stomatal conductance and aperture measurements. Bimolecular fluorescence complementation, luciferase and GFP-based protein accumulation assays were conducted to investigate if SLs modulate LA and PRO at the protein level. Our experiments showed that mutants impaired in miR319-mediated degradation of the LA transcript or in PRO expression do not react to SL treatment by reducing stomatal conductance and aperture as wild type plants do, indicating that the miR319–LA module and PRO are required for SL-triggered stomatal movement. The reporter assays provided evidence that LA protein abundance is reduced by SLs, and this effect is exerted at the transcript level via miR319-mediated degradation. Together, these results provide evidence that SLs regulate stomatal movement through the miR319–LA and/or PRO in tomato. To this end, we are producing additional single and double mutants of the relevant genes, and devising new assays to explore the stability and interaction with PRO of a LA protein encoded by miR319-insensitive mRNA.

Role of thallium hyperaccumulation in plant protection against biotic stress: insights from *Silene latifolia* and *Botrytis cinerea*.

Chiavacci Beatrice*, Baccelli Ivan, Martinelli Federico, Colzi Ilaria, Gonnelli Cristina*

University of Firenze

Metal-hyperaccumulation is an evolutionary trait found in over 700 plant species and generally explained by the “elemental defense” hypothesis, strongly supported by studies involving herbivorous insects. In contrast, limited research, only on zinc and cadmium, has investigated the effects of hyperaccumulation on plant pathogens.
Our research aimed to understand whether thallium-hyperaccumulation (Tl) prevents the pathogenic attack of *Botrytis cinerea* in the facultative hyperaccumulator *Silene latifolia*, comparing a metallicolous population (Saint Laurent-le-Minier, France) with a non-metallicolous one (Barraux, France). Plants (6 replicates for each treatment) were cultivated for 3 weeks in hydroponic solution with increasing Tl concentrations (0, 2.5, 5μM TlNO3) in a growth chamber. The infection with conidial suspension of *B. cinerea* was performed on 3 different leaves per plant and the necrotic area was tracked over time as index of plant susceptibility to the pathogen. Biometric and physiologic parameters and ionome were evaluated at the end of the experiment.
Thallium exposure did not affect the growth of the metallicolous population of *S. latifolia*, therefore considered as tolerant, whereas the non-metallicolous one showed severe toxicity symptoms. The lesion area caused by the pathogen was larger in the tolerant population compared to the sensitive one, showing a proportional increase in necrotic region diameter with increasing Tl concentrations. The unexpected higher susceptibility of the hyperaccumulating population could be attributed to multiple factors. The level of Tl accumulated by the shoot (~1500 μg g^{-1}) was not enough to inhibit the development of the pathogen. In the non-metallicolous population Tl-toxicity (~400μg g-1) did not allow a normal leaf development and consequently the expansion of the infection. The higher stomata density, developed by the Tl-treated hyperaccumulating population, probably promoted *B. cinerea* infection.
To better understand the role of Tl-hyperaccumulation in defense against plant pathogens, *S. latifolia* plants will be exposed to higher Tl concentrations for a longer period to achieve metal accumulation comparable to those observed in plants grown in metalliferous soils (up to 80000 μg g^{-1}) before infection.

Priming-induced DDR (DNA Damage Response) signatures contributing to heat stress tolerance in tomato (*Solanum lycopersicum* L.)

Ridwan Diaguna *(1), Alma Balestrazz (1)i, Anca Macovei (1), Massimiliano Beretta (2)*

(1) University of Pavia
(2) Panora S.p.A, Fidenza

Tomato is a widely grown vegetable, relevant for human consumption, yet recurrent heat waves place its production at risk. Pre-sown treatments (seed priming) can mitigate high-temperature damage by enhancing the protective mechanisms activated during the seed pre-germinative metabolism. As seed vigour relies on effective DNA repair and antioxidant responses triggered during early imbibition, a deeper understanding of the molecular processes underlying germination is essential for developing more effective priming techniques. The aim of this work is to design optimised, sustainable priming protocols for tomato seeds, minimizing the influence of genotype and seed lot, able to improve heat stress tolerance. Eleven tomato genotypes are being currently screened for heat tolerance to select heat-sensitive and heat-tolerant genotypes for priming optimization. Hydropriming, chemo-priming, and physical priming will be assessed for testing specific parameters to generate optimized protocols. Their impact on heat stress tolerance will be evaluated through standard germination tests. Primed and unprimed seeds of sensitive and tolerant tomato genotypes will be analysed in presence/absence of heat stress whereas molecular profiling will include quantification of reactive oxygen species (ROS) and DNA damage, metabolomics, RNA-Seq and ATAC-Seq. These tasks will be achieved in the long-term in the frame of the HeatDDR project (https://www.heatddr.eu/). The results of the study will reveal DDR signatures associated with heat stress tolerance triggered by seed priming and the related regulatory networks. The selected seed-priming protocols will be applied to a wide range of genotypes to identify the most sustainable treatments, showing minimal genotype-dependent effects, combined with low cost, and limited environmental impact.

An Integrative Multi-Omic Approach to Decode Adaptive Strategies of *Atropa belladonna* in Saline Environments

***D'Alessandro R.**, Santoro V., Principio L., Cimmino L., Cirillo V., D'Amelia V., Esposito S., Piccinelli A.L., Rastrelli L., Docimo T.*

Università degli Studi di Salerno (UNISA)

Atropa belladonna L., an invasive species native to Europe, North Africa, and Western Asia, is increasingly spreading into agroecosystems, with potential food-safety implications related to the presence of tropane alkaloids (TAs). Its ability to colonize marginal environments and withstand adverse conditions, including soil salinization intensified by climate change, makes this species a valuable model for investigating mechanisms of abiotic stress adaptation. In this study, we conducted physiological, biochemical, metabolic, and transcriptional analyses to investigate the responses of *A. belladonna* exposed to salt stress (80 and 150 mM NaCl for 21 days) under controlled greenhouse conditions. Physiological measurements, including photosynthetic efficiency and stomatal conductance, indicated stress symptoms only at the highest salt concentration, confirming the species' tolerance to salinity. Biochemical analyses conducted on above and belowground tissues revealed root accumulation of proline and phenolic compounds, and low malondialdehyde (MDA) levels in both tissues suggested limited lipid peroxidation and effective control of oxidative stress. Transcriptomic profiling revealed gene-expression reprogramming in response to salinity, with tissue specific patterns emerging within hormonal signaling networks involving increase in ethylene, jasmonic acid (JA), and abscisic acid (ABA). Metabolomic data supported these findings, showing hormone fluctuations consistent with transcriptional trends, namely a significant increase in JA in roots and of ABA and salicylic acid in leaves, respectively. Although upon stress, TA levels remained stable in the whole plant, genes involved in biosynthesis of polyamine, which are also TA precursors were consistently upregulated in root tissues, suggesting their potential involvement in maintaining carbon–nitrogen homeostasis under salt stress. Overall, the results reveal a coordinated molecular and biochemical strategy enhancing *A. belladonna* fitness in saline environments and contributing to its invasive potential. Linking TA-related metabolism to salinity tolerance provides insight into traits underlying resilience and may inform development of salt-tolerant crops and strategies to limit the spread of this species.

Investigating stress memory in tomato plants exposed to recurrent water deficit

G. Giannetti *(1) *, A. Moine (1), C. Morabito (2-3), F. Secchi (2), C. Pagliarani(1)*

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(3) Department of Biology, University of Padova, Via U. Bassi 58/b, 35131 Padova, Italy

Keywords: eco-physiology, drought priming, recovery, transcriptome reprogramming

Drought is one of the climate-change enhanced stresses that most critically alter crop productivity in the Mediterranean basin. Deciphering the biological bases of plant adaptation to water scarcity is instrumental to improve the resilience of crop management systems. Though tomato physiological and transcriptional responses to single drought and rehydration events were investigated, drought-primed ecological memory is still an underexplored research matter.
To study whether repeated water deficit exposure could prime drought tolerance, tomato plants were exposed to a first water deprivation treatment (WS) followed by recovery. A second WS treatment was then applied to the same plants (PRIMED) and to another group previously irrigated (UNPRIMED). Well-watered controls (CTR) were also included in the trial. Biometric, anatomical and physiological traits were measured during both the WS and recovery time courses, while leaves and roots were collected at the end of the second WS experiment for metabolic and molecular analyses. PRIMED plants showed improved transpiration and assimilation rates than the UNPRIMED ones. Priming-dependent effects also emerged from the analysis of chlorophyll content and stomatal density, which were significantly higher in PRIMED than UNPRIMED plants, regardless of WS.
Metabolic data revealed that accumulation of soluble carbohydrates and osmolytes did not significantly differ in leaves and roots of all plant groups independently of WS, while starch content was opposite in the leaves of PRIMED and UNPRIMED plants based on the water regime.
RNA-seq elaboration indicated that the transcriptome of tomato plants exposed to only one input of WS and then rehydrated was not significantly perturbed compared to that of CTR plants, suggesting a reset of stress signals at the molecular level. Integration of these data with the analysis of DNA methylome marks in PRIMED and UNPRIMED plants is ongoing to better elucidate the establishment of stress memory signals.

This work was supported by the REMIND project funded by the European Union through the Next-Generation EU programme [Piano Nazionale di Ripresa e Resilienza (PNRR), Missione 4 ''Istruzione e Ricerca'', Componente C2 Dalla ricerca all'impresa, Investimento 1.1 ''Fondo per il Programma Nazionale della Ricerca (PNR) e Progetti di Ricerca di Rilevante Interesse Nazionale (PRIN)2'', CUP B53D23018020006, Grant number 2022RBHRJR)].

Trophic transitions from dark to light in the unicellular red alga *Galdieria phlegrea*

Rosanna Bossa, Francesco Loreto, Giovanna Salbitani, Simona Carfagna

University of Naples, Federico II

Galdieria phlegrea (strain 734) is a unicellular and polyextremophilic red microalga, endowed with high metabolic flexibility, which allows it to alternate trophic modalities (autotrophic, heterotrophic, and mixotrophic) and to thrive in extreme environmental conditions.
The present study investigates the physiological and functional mechanisms of photosynthetic reactivation during the trophic transition from heterotrophy to autotrophy, to define temporal dynamics, reorganization of photosystems, and strategies of use of intracellular reserves.
The results show that heterotrophic cells accumulate energy reserves in the form of lipids and carbohydrates, keeping the functional core of the photosystems unchanged. Cell density increases, while cell size decreases in the transition to autotrophy. Exposure to light initiates a coordinated process of reactivation of photosynthesis. In fact, following the transition in five days, it is observed that the maximum quantum yield of Photosystem II (Fv/Fm), together with the effective quantum yield of Photosystem II (ΦPSII), increases within five days, indicating the restoration of electronic transfer and oxygen production. The results are confirmed by spectroscopic, 77K fluorescence, and confocal microscopy analyses, which show that Photosystem I (PSI) maintains structural and functional integrity even in the absence of light, acting as a center for reorganization, while the antennal complexes and Photosystem II (PSII) recompose rapidly.
In conclusion, we can argue that photosynthesis recovery takes place in three distinct phases: (i) latency; (ii) activation, and (iii) stabilization, optimizing the use of stored energy resources, which reflect an optimization of the deployment of intracellular energy reserves.
These results highlight the exceptional resilience and versatility of *G. phlegrea* and suggest its potential as a model organism for trophic adaptation studies and for biotechnological applications.

Understanding the impact of photoprotection mechanisms on ROS regulation in the moss *Physcomitrium patens*

Francesco Paolo Tranne(*1), Claudia Beraldo(1), Alessandro Alboresi(1) and Tomas Morosinotto(1)*

(1) Università di Padova

To meet the increasing global demand for food, agricultural yields will need to rise by an estimated 60–110% by mid-century, while simultaneously reducing the environmental impact of agriculture. A new agricultural revolution is needed, one that harnesses cutting-edge genetic technologies with a particular focus on improving photosynthetic efficiency. Photosynthesis converts light into energy and represents the fundamental biochemical pathway that sustains plant life and other photosynthetic organisms. Regulatory mechanisms such as cyclic electron flow (CEF) and pseudo-cyclic electron flow (PCEF) play a key role in modulating photosynthetic electron transport and are essential for photoprotection under high and fluctuating light conditions, with a significant impact on growth [1,2]. We hypothesised that this is associated with the uncontrolled accumulation of reactive oxygen species (ROS), resulting in photodamage to photosystem I (PSI). This study aims to elucidate the role of CEF and PCEF in mitigating ROS accumulation by quantifying ROS production in *Physcomitrium patens* mutants lacking one or both photoprotective mechanisms and exposed to either constant high light or a fluctuating light regime. We also aim to investigate the impact of ROS accumulation on the redox regulation system of P. patens. By comparing these mutants to the WT, we expect to observe higher ROS levels in CEF or PCEF KO mutants followed by a significant impairment in Calvin–Benson cycle regulation.

Secretion and Regulation of Pectin Methylesterases as Mechanisms of Plant Adaptation to Environmental Stress

Pecatelli Gabriele *(1), De Caroli Monica (2) and Lionetti Vincenzo (1)*

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The plant cell wall is a dynamic and tightly regulated structure that is essential for plant growth, development, and adaptation to environmental challenges. Among the enzymes involved in cell wall modification, pectin methylesterases (PMEs) regulate the degree of homogalacturonan methylesterification, thereby influencing wall mechanical properties and cell signaling processes (Lionetti 2015; Lionetti et al. 2017). Although PME activity has been associated with stress responses (Coculo et al. 2023), the precise roles of individual isoforms remain largely unclear. In this study, we investigated PME gene expression in Arabidopsis thaliana under different environmental stress conditions, including temperature extremes, water deficit, salinity, and biotic challenges. Our findings indicate that specific PME isoforms participate in distinct plant responses to environmental stimuli. Notably, these isoforms are delivered to the cell wall through multiple secretory routes, encompassing both conventional and unconventional pathways. Such diversity in trafficking mechanisms may determine the spatial and temporal regulation of PME activity, ultimately shaping the plant's ability to remodel its cell wall during stress. The implications of PME-driven cell wall remodeling in plant adaptation to diverse environmental stresses are discussed.

Coculo D, et al 2023. Plant Physiology and Biochemistry. 201:107865.
Lionetti V. 2015. Front Plant Sci. 6:331.
Lionetti V, et al 2017. Plant Physiol. Jan 12.

Transcriptomic and metabolic reprogramming of durum wheat under polystyrene nanoplastics exposure

Benedetta Pizziconi

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Polystyrene nanoplastics (PSNPs) in agricultural soils threaten crop productivity and animal health, including humans, by potentially entering the agri-food chain. Using high-throughtput bioimaging techniques, molecular and biochemical analysis, the study aimed to investigate entrance and translocation of PSNPs in durum wheat, and their consequent effects on crop growth, metabolism and transcriptomic profile. The study involved two genotypes, Kronos and MRP3, a tillering mutant for the TdMRP3 gene, that encodes a phytic acid vacuolar transporter also responsible for xenobiotics vacuolar sequestration. Kronos and MRP3 were hydroponically exposed to 10 mg/L PSNPs for 21 days. PSNPs penetrated roots and translocated to shoots. PSNPs impaired the mutant's growth, while promoting Kronos'. In both genotype, PSNPs increased glucose and fructose, indicating a shift toward rapid energy mobilization. Sucrose, an osmolyte and storage sugar, only increased in Kronos, suggesting an adaptive mechanism to balance this metabolic shift. RNA sequencing revealed osmotic stress responses in both genotypes, with upregulation of genes for aquaporins in Kronos and for proline in MRP3. The mutant also displayed a strong photosynthetic repression coupled with reduced photosynthetic efficiency, electron transport, gas water exchange and transpiration rate. Kronos only showed a reduction in the electron transport and transpiration rate, while promoted photosynthetic efficiency and gas water exchange. Despite no visible detrimental effects on Kronos' phenotype, PSNPs profoundly altered both genotypes metabolism and transcriptomic profile. Remarkably, PSNPs exposure caused transpiration decifit and osmotic stress in Krons and MRP3, alerady at 21 days of exposure. Collectively, results highlight early markers of PSNPs-induced stress in both genotypes, indicating ongoing damage that may lead to yield losses, prolonging the exposure. Addittionally, PSNPs translocation from roots to above sites of durum wheat, rises food serious safety concers.

Session 2: Plant Nutrition and Metabolism

Phosphoribulokinase: how to torment an enzyme to study carbon metabolism

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Protein studies provide from atomic to physiological information. In particular, research on enzymes involved in CO_2 fixation metabolism in photosynthetic organisms, that is, the Calvin-Benson-Bassham cycle, has provided clues on the catalytic properties, regulatory features, molecular interactions, and potential bottlenecks. In recent years, our laboratory has studied phosphoribulokinase (PRK), the last enzyme of the CO_2 fixation cycle, which regenerates ribulose-1,5-bisphosphate, the substrate of Rubisco. To understand the evolution of its regulation and catalysis, PRKs from the cyanobacterium Synechococcus sp. PCC 6803, the green alga *Chlamydomonas reinhardtii*, and the angiosperm *Arabidopsis thaliana* have been expressed recombinantly. Distinct features and common trends emerged analysing the influence of physiological parameters, e.g., pH variation, and the regulation by thioredoxins (TRXs). The latter constitutes one of the main mechanisms to tune the activity of CO_2 fixation enzymes depending on presence and intensity of light. Recently, discovery of the crystallographic structure of PRK explained the molecular basis of TRX regulation and the mechanism for its inhibition.
Despite these new details, the driver for PRK-TRX interaction remain largely unknown. Sequence analysis identified the insertion of a loop in eukaryotic PRK, the Clamp loop, localized just above the regulatory elements involved in TRX regulation. The deletion of the Clamp loop highlighted a critical influence on redox sensitivity and a partial role in TRX recognition.
Going beyond the characterization, our latest experiments focused on the rational design of a PRK variant with improved affinity for its sugar substrate, aiming to increase the flux of CO_2 fixation cycle. Structural analysis, molecular dynamics, and kinetic studies led to a first PRK mutant to be further improved with new rounds of design and testing in vivo.
Overall, our work shows the many perspectives from which an enzyme like PRK can be approached to disentangle different biochemical and physiological aspects of its function.

Heterologous expression of phosphoenolpyruvate carboxylase in *Nannochloropsis oceanica*

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Microalgae are photosynthetic unicellular microorganisms that are well known to produce high value molecules that are suitable in feed, food and cosmetics. Despite their advantages compared to land plants, the productivity potential of microalgae remains untapped and the biomolecules production at industrial scale level is still poorly competitive. Domestication of microalgae is necessary to improve the growth in photobioreactors to overcome the costs of the biomass production of these microorganisms.

Microalgal growth is regulated by interplay of carbon fixation (Calvin Benson Bassham cycle) and respiration (Tricarbossilic Acid cycle). Study of carbon fluxes and introduction of alternative mechanisms to improve fixation of CO_2 or respiration can boost photosynthesis and enhance biomass production.

PhosphoEnolPyruvate Carboxylase (PEPC) is an enzyme commonly present in C4 and C4-like plants. It has the function of fixing CO_2 catalyzing the carboxylation of phosphoenolpyruvate to oxalacetate and it is already expressed in *N. oceanica* mitochondria. Different studies on land plants showed an improvement on drought stress and photosynthetic parameters when this enzyme is overexpressed.

The aim of the project is to evaluate the potentially beneficial overexpression of PEPC from *M. thermautotrophicus* in *Nannochloropsis oceanica*, and to study the primary carbon fluxes in this microalga.

PEPC chloroplastic and cytosolic overexpressors were obtained and their phenotype analyzed in terms of biomass production in lab scale photobioreactor in presence of atmospheric or enriched CO_2 air. The strains obtained show slower growth, the main differences in growth were observed in condition of high CO_2 availability. Photosynthetic parameters were also measured to assess the growth phenotype causes. Metabolic profiling will be conducted, and an existing metabolic model will be evaluated.

These results offer a deeper understanding of carbon fluxes in *N. oceanica* and the interplay between different organelles and metabolism in this host.

Iodine: a new weapon in the battle against abiotic stress

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Iodine has recently been recognized as a plant nutrient, with increasing evidence suggesting it may enhance tolerance to abiotic and biotic stresses. However, previous studies have often been conducted on different species, under varying growth conditions, and with diverse experimental setups, resulting in fragmented and difficult-to-compare findings. The aim of this work was to evaluate the potential protective role of iodine against multiple abiotic stresses, using a standardized experimental system to generate comparable results.

The model species *Arabidopsis thaliana* was grown in a hydroponic floating system and subjected to a short, three-day iodine pre-treatment at different KI concentrations (0, 10, and 30 μM), before being exposed to salinity, heat, or drought stress. This uniform setup allowed a direct assessment of iodine-induced effects across different stress conditions.

Despite the brevity of the treatment, iodine pre-exposed plants showed a marked reduction in mortality and attenuation of stress-related physiopathies under all tested conditions. Consistently, RT-qPCR analyses showed a strong upregulation of stress-responsive marker genes in iodine pre-treated plants.

This study demonstrates that a short and transient iodine pre-treatment elicits a conserved priming response that enhances tolerance to a broad range of abiotic stresses and points to the promising opportunity to extend these findings to economically important crops, such as tomato and lettuce.

Investigation of the metabolic coordination in diazotrophic cyanobacteria using genetically encoded fluorescent probes.

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Diazotrophic cyanobacteria are characterized by the capability to perform both oxygenic photosynthesis and nitrogen fixation, despite the two processes being functionally incompatible, as the latter is inhibited by oxygen. To overcome this limitation, the filamentous cyanobacterium *Anabaena* sp. PCC7120 differentiates a fraction of its photosynthetic vegetative cells (VCs) into nitrogen-fixing heterocysts (HCs) when grown in absence of combined nitrogen. Given the importance of both nitrogen and carbon for cell survival and growth, VCs and HCs must coordinate the two assimilation pathways dynamically, according to their metabolic needs. Indeed, nitrogen and carbon metabolisms are strictly interdependent and must share regulatory feedback influencing one according to the state of the other: to investigate this mutual coordination , a set of genetically encoded fluorescent biosensors sensitive to physicochemical parameters (i.e. redox state and pH) and to the concentration of carriers of chemical energy and reducing equivalents (i.e. ATP and NADPH) is being used to achieve the spatial and temporal resolution required to measure how the metabolism of both cell types differs from each other's and responds to environmental cues in real time. To date, the sensors allowed to show that intracellular pH is significantly influenced by different light regimes, and that HCs and VCs respond to actinic light in a similar way but the former maintain a higher intracellular pH. The entire set of probes will be used to further dissect the physiological differences and responses of both cell types, in order to interpret their metabolic dynamics, providing insights for targeted engineering and future exploitation of the biological system in biotechnology (e.g. as biofertilizer in agriculture).

Physiological and Molecular Effects of Exogenous Strigolactone-like Molecules in Nutrient Use Efficiency in Tomato

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Frequent extreme weather events increasingly stress crops, reducing productivity. Adequate nutrient supply—especially nitrogen—is essential for healthy plant growth. The phytohormones strigolactones play important roles in plant acclimation to abiotic stress and in improving nutrient use efficiency (NUE). Karrikins (KARs), strigolactone-like compounds derived from smoke of burning vegetation, are perceived by the KAR INSENSITIVE2 (KAI2) receptor and can enhance tolerance to several abiotic stresses.
This study aims to identify the physiological and molecular mechanisms regulating NUE in tomato (*Solanum lycopersicum*) under normal and nutrient-deficient conditions, as influenced by KARs. We conducted hydroponic experiments with tomatoes grown under varying nitrogen levels and treated with KAR1, "smoke water" (KAR-enriched), or water. Under nitrogen-deficient conditions (3.38 mM), both KAR1 and smoke water increased root NUE, and smoke water also enhanced leaf NUE. In contrast, under nitrogen-sufficient conditions (13.3 mM), smoke water reduced root NUE. To further elucidate how KAR signalling affects NUE, we employed virus-induced gene silencing (VIGS) to simultaneously target the putative KAR receptor paralogues KAI2a, KAI2b, and KAI2c. Silencing was achieved in both leaves and roots, with optimal efficiency maintained for at least three weeks. This system will be used to determine whether the effects of KARs on morphology and stress signalling depend on KAI2, and also if the endogenous KAI2 ligand KL (of yet unknown nature) has a role in resource allocation and use efficiency in tomato. We will assess morphology and quantify the expression of key genes in nitrogen uptake and transport in mock- or KAI2-silenced plants subjected to nitrogen-replete or -depleted conditions, with or without KAR1 or smoke water treatment.

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Re-Waste: a green, sustainable and circular strategy for crop resilience

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In the EU, an estimated 20% of the total food produced is lost or wasted. Food waste is rich in biomolecules, able to cause changes in crop important physiological processes. The purpose of the current study is to exploit waste by-product to develop a sustainable strategy for crop plant growth and resilience to (a)biotic stresses. We, thus, conducted agronomic trials on two model species for agriculture (wheat and tomato) by using agro-food waste, that was compacted and dehydrated, and the solid dry residue (SDR) obtained was directly added to growth substrate. These SDRs represent a potential reservoir of bioactive products, including oligosaccharins, such as chitooligosaccharides and oligogalacturonides, both potential alternative to traditional agrochemicals. We propose here a novel, safe and sustainable strategy based on a model that promotes circular economy by recycling wet-organic waste, replacing chemical fertilizers, reducing pollution and high management costs.

Recombinant production and characterisation of chloroplastic pentose phosphate pathway dehydrogenases from *Chlamydomonas reinhardtii*

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The oxidative pentose phosphate pathway (OPPP) plays a fundamental role in plant cells. Despite its well-known role in Eukarya providing ribulose-5-phosphate (Ru5P) and NADPH for primary metabolism, in plants this enzyme is present in different isoforms (cytosolic, chloroplastic, plastidial and peroxisomal). These different enzymes are involved in several processes, from development to stress response.
The first oxidative steps of this pathway provide a large amount of NADPH through reactions catalysed by two key enzymes: glucose-6-phosphate dehydrogenase (G6PDH), which converts glucose-6-phosphate into 6-glucono-δ-lactone, and 6-phosphogluconate dehydrogenase (6PGDH), which catalyses the oxidative decarboxylation of 6-phosphogluconate to Ru5P.
Although these enzymes have been studied in higher plants, microalgal systems remain largely unexplored. To address this gap, this project aims to characterise the chloroplast-associated OPPP dehydrogenases of *Chlamydomonas reinhardtii.*
Both CrP1 G6PDH and CrP1 6PGDH have been heterologously overexpressed and purified from Escherichia coli, yielding catalytically competent recombinant enzymes. Their catalytic behaviour has been investigated through an accurate kinetic characterisation, including the determination of Michaelis–Menten parameters, substrate preferences, and cofactor dependencies. Regulatory aspects of CrP1 G6PDH have also been investigated, revealing redox-sensitive features that may contribute to the modulation of the OPPP under physiological conditions. A comparable experimental framework is currently being applied to CrP1 6PGDH to determine its possible regulatory mechanism(s). Furthermore, the potential interaction between CrP1 G6PDH and CrP1 6PGDH will be assessed to explore whether these enzymes may form a functional multi-enzyme assembly within the chloroplast, which could support spatial organisation or substrate channelling within the microalgal OPPP.
Overall, this study provides the first detailed biochemical characterisation of chloroplastic OPPP dehydrogenases in microalgae, contributing new insights into the functional organisation and regulatory architecture of this essential metabolic pathway.

Structural determinants of functional and regulatory features of photosynthetic phosphoglycerate kinase

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Phosphoglycerate kinase (PGK; EC 2.7.2.3) occupies a pivotal position in the Calvin-Benson-Bassham cycle by catalyzing the reversible, Mg^{2+}-dependent transfer of a phosphoryl group from ATP to 3-phosphoglycerate (3-PGA), yielding 1,3-bisphosphoglycerate (BPGA) and ADP. In chloroplasts, photosynthetic PGK operates within light-responsive stromal metabolism through a conserved two-domain architecture that undergoes hinge-bending conformational changes essential for catalysis. Here, we investigate the photosynthetic PGK1 from the green alga *Chlamydomonas reinhardtii* (CrPGK1), a redox-regulated enzyme controlled by conserved cysteine residues. Kinetic analyses revealed high catalytic efficiency of the wild-type enzyme, whereas single-cysteine variants (C227S or C361S) displayed distinct effects on turnover and substrate affinity, indicating residue-specific roles in conformational transitions linked to domain closure. Crystal structures of the reduced apo enzyme and of complexes with 3-PGA and ADP captured discrete ligand-induced conformational states, while molecular dynamics simulations showed that Cys227 and Cys361 differentially modulate hinge flexibility and inter-domain communication. Moreover, a structural mapping of putative redox- and phosphorylation-dependent post-translational modifications was carried out on the foundation of crystallographic findings. Together, these results establish a structural framework linking conformational dynamics to the regulation of PGK activity and identify chloroplast CrPGK1 as a finely tuned enzyme that integrates stromal ATP availability, metabolite levels and light-dependent signals to optimize photosynthetic carbon assimilation.

Session 3: Development and signal transduction

Keeping Hormones in Check: Homeostatic Control of Auxin and Jasmonate Action

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Auxin and jasmonates (JAs) are central regulators of plant morphogenesis and immunity. Despite their structural and functional differences, both hormones are perceived and transduced in the nucleus through homologous protein complexes. The compartmentalized localization of auxin- and JA-related enzymes further suggests that distinct cellular compartments contribute to controlling hormone homeostasis.

Recent discoveries show that auxin perception is not restricted to the nucleus but also occurs in the cytoplasm and extracellular space, emphasizing the importance of intracellular regulation in shaping cellular and organellar responses. However, the exact mechanisms controlling the distribution of auxin and JAs within the cell remain largely unexplored. Both hormones need to be regulated before a cell can respond to a new stimulus, a process mediated by enzymes such as GH3s, 2-oxoglutarate/Fe(II) dioxygenases, and IAA-Leu-Resistant 1 (ILR1) and ILR1-like (ILL) amidohydrolases.

We have demonstrated that auxin-inactivating enzymes, including GH3s and DIOXYGENASE FOR AUXIN OXIDATION (DAO), localize to both the cytosol and nucleus, supporting a role in fine-tuning intracellular auxin perception. Under stress conditions, GH3 enzymes also conjugate amino acids to *cis*-(+)-12-oxo-phytodienoic acid (*cis*-OPDA), a JA precursor that functions as an independent stress signal and may contribute to climate adaptation. OPDA-aa are subsequently hydrolyzed by ILR1/ILL amidohydrolases in the endoplasmic reticulum, marking a potential regulatory step in the pathway as the release of free *cis*-OPDA into the nucleus may influence its signaling function.

Isolation of extracellular vesicles and characterization of their role as shuttle of biologically active small RNAs in intercellular communication

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Extracellular vesicles (EVs) are nanoscale particles delimited by a lipid bilayer membrane, secreted by most eukaryotic cells into the extracellular space. Several evidences support the crucial role played by EVs in cell-to-cell communication as carriers of functional biological molecules, such as small non-coding RNAs (sRNAs). In plants, as part of the infection process, a communication mechanism called bi-directional cross-kingdom RNA interference occurs, based on sRNA trafficking via EVs between fungal pathogens and host plants. Therefore, EVs have emerged as nanovectors of genetic information, and the study of their composition and biological properties in different plant species is a field of research in rapid expansion.
The aim of this research is the optimization of a protocol for the isolation of EVs from apoplastic washing fluid collected from *Nicotiana benthamiana* leaves. The final goal is the characterization of the small RNA composition of EVs by comparing samples isolated from wild-type plants with those from plants expressing an antiviral construct against the Grapevine Fan Leaf Virus and the Grapevine Leaf Roll-associated Virus. Differential centrifugation was employed to isolate EVs, and the sedimented material was physically characterized by TEM and DLS. We obtained EVs with an average diameter of 100-150 nm. After treatment with RNAse to remove RNA potentially present outside the vesicles, we proceed with EVs lysis and isolation of the sRNAs. RNA sequencing will be performed to characterize sRNAs present in the EVs samples.
The results will have both heuristic relevance, increasing knowledge beyond state of the art concerning the movement of the silencing signal in plants, and applied interest, for exogenous application, using EVs as shuttles of sRNAs for improving crop protection and silencing of target genes.

The role of HPCA1 and GLR3.3 in ROS perception and long-distance signal propagation after wounding in *A. thaliana* seedlings

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Plants have evolved sophisticated long-distance signaling networks to coordinate systemic responses to environmental stress. Beyond hormones and metabolites, the integration of signals such as electrical waves, hydraulic wave, reactive oxygen species (ROS), and calcium (Ca^{2+}) waves is crucial for cell-to-cell communication. In this study, we investigated the roles of HPCA1, a plasma membrane H_2O_2 receptor that triggers cytosolic Ca^{2+} increases, and GLR3.3, a glutamate receptor-like channel that also leads to cytosolic Ca^{2+} influx, in the systemic response to mechanical wounding in *Arabidopsis thaliana* seedlings. In wild-type (WT) seedlings, mechanical wounding prompts a highly coordinated stomatal closure, affecting both wounded (local) and unwounded (distal) tissues. In contrast, hpca1 mutants failed to exhibit wound-induced stomatal closure, suggesting that HPCA1 is essential for the long-distance signal propagation and for the translation of this signal into physiological responses. Live imaging of guard cells using the CM-H2DCFDA probe revealed that, while WT stomatal guard cells show robust ROS accumulation following wounding, hpca1 guard cells failed to exhibit significant increases in ROS-associated fluorescence. In these mutants, ROS levels remained comparable to control conditions at all analyzed time points, except for a minor, transient spike 5 minutes after root wounding. Furthermore, analysis of in vivo ROS accumulation in whole cotyledonary leaves of hpca1 and glr3.3 mutants revealed that these mutant seedlings did not display ROS accumulation in their local or systemic leaves in response to cotyledonary-leaf or root wounding, suggesting that both proteins are indispensable for wound-triggered cell-to-cell ROS signal propagation. Collectively, our findings identify HPCA1 and GLR3.3 as key components of a ROS-Ca^{2+} signaling that couples local perception of wounding to systemic acclimation, providing new insights into the crosstalk between ROS accumulation and Ca^{2+} fluxes during cell-to-cell signal propagation.

Dissecting Oligogalacturonide-Mediated Pathogen Resistance and Root Development

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Oligogalacturonides (OGs) are damage-associated molecular patterns (DAMPs) generated during cell wall pectin degradation and are well-established signaling molecules in plant immunity and development. Their production depends on the interplay between polygalacturonases (PGs), which hydrolyze homogalacturonan, and PG-inhibiting proteins (PGIPs), which modulate OG accumulation towards the size classes that most strongly activate immune responses.
To study OG function in planta with temporal control, we generated transgenic *Arabidopsis thaliana* lines expressing a β-estradiol-inducible PGIP-PG chimera ("OG machine," OGM) that enables on-demand OG production. Upon induction, OGM plants display enhanced resistance to microbial pathogens and elevated salicylic acid (SA) levels, but also show root growth inhibition and reduced biomass, consistent with a role for OGs in the growth–defense trade-off.
To dissect the molecular mechanisms underlying these phenotypes, we expressed OGM in mutant backgrounds defective in key immune components. Using these crosses, we show that OGM-induced root growth inhibition requires the NADPH oxidase RBOHD and JAR1, which is necessary for synthesis of bioactive jasmonate, and depends largely on the immune regulator EDS1. In contrast, this phenotype is independent of SA and other factors such as NDR1, and does not require MPK3 or MPK6 individually. Notably, the enhanced resistance of OGM plants to the necrotrophic fungus *Botrytis cinerea* is JAR1-dependent, but does not require RBOHD or EDS1. Together, these results indicate that OGs engage distinct hormone-linked signaling modules to differentially regulate immunity and development. Ongoing transcriptomic analyses will further clarify the pathways activated downstream of OGs that lead to resistance to *B. cinerea.*

NADKinases-Calmodulin-dependent as driver for pollen tube tip growth

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NADP(H) is obtained by means of specialised enzymes that can phosphorylate NAD(H), named NAD-Kinases (NADKs). In *Arabidopsis thaliana* four NADK isoforms exist, one of which is activated upon Calmodulin (CaM). This class of NADKs has been named NADK-CaM-dependent (NADKc) and is present only along the green lineage. In *Arabidopsis* seedlings, it has been demonstrated that NADKc1 acts upstream NADPH oxidases (RBOHs), affecting the production of Reactive Oxygen Species (ROS) in response to the bacterial elicitor flagellin22.
Notably, RBOH-generated-ROS and Calcium are key players for the regulation of tip growth processes. Pollen tube tip growth relies on their concertation, that if disrupted causes abnormal pollen tube growth and even infertility. However, the extent to which NAD(P)/H and NADKs participate in such process has never been investigated in detail.
Here we display that two newly discovered putative NADKcs of *Arabidopsis thaliana*, named NADKc2 and 3, have a role in the pollen tube development and fertilisation success.
We show that NADKc2 and NADKc3 are exclusively expressed in mature pollen grains and pollen tubes. While single mutants were mildly affected, the double mutant showed both in vitro and in vivo reduced pollen tube elongation. Microscopy inspection of nadkc2/nadkc3 pollen tubes showed bulged tips, compared to the wild type. Importantly, the double mutant had reduced silique length and seed number, compared to the wild type. Our results suggest that the altered pollen development of the mutant, has an impact on fertilization success, that was even more affected upon a mild heat stress.
The discovery that NADKcs are important for the fertilization process has then motivated us to study if their role is conserved evolutionary focusing on the bryophyte Marchantia polymorpha. We show that Marchantia has only one NADKc homolog, which is expressed in both reproductive and vegetative tissues, raising important questions on the diversification of NADKcs.

Dissecting the Mitochondrial Unfolded Protein Response (UPRmt): From Stress Signaling to Physiological Acclimation in *Arabidopsis*

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Mitochondria, in addition to being the primary powerhouse of the plant cell and essential hubs for multiple metabolic processes, also function as environmental sensors capable of perceiving the stress and orchestrating adaptive cellular responses. Maintaining mitochondrial protein homeostasis is crucial for proper organelle function and is primarily ensured by the mitochondrial protein quality control (mtPQC) system, a complex network of chaperones and proteases responsible for eliminating misfolded or damaged proteins and preventing proteotoxic damage inside the organelle. When protein damage exceeds the capacity of the mtPQC, compensatory pathways are activated, including the mitochondrial unfolded protein response (UPRmt). UPRmt is a retrograde signaling pathway that promotes the expression of nuclear-encoded mitochondrial chaperones and proteases to reinforce the mtPQC network. Although UPRmt has been identified in various eukaryotic systems, the regulatory mechanism of plant UPRmt remain poorly understood. In *Arabidopsis thaliana*, UPRmt activation has been associated with a transient oxidative burst followed by a MAP kinase–mediated signaling cascade, and appears to intersect with hormone signaling pathways, particularly ethylene, auxin and jasmonate. Recent transcriptomic analyses in Arabidopsis have identified new candidate genes strongly induced during mitochondrial proteotoxic, potentially serving as UPRmt markers. This project aims to identify new molecular components of the plant UPRmt pathway and investigate its physiological role in abiotic stress resistance. To this end, we will combine *Arabidopsis* mutant lines with constitutive UPRmt activation and altered mitochondrial proteostasis (e.g. protease and ribosomal mutants) with loss of function lines for candidate signaling components, together with chemical induction of mitochondrial proteotoxic stress. Mitochondrial morphology and dynamics, Ca^{2+} signaling and stress marker gene expression, as well as the physiological and molecular characterization of these mutant backgrounds, will be analyzed. In parallel, physiological and molecular phenotyping under selected abiotic stress conditions will be performed in order to evaluate the contribution of UPRmt and its candidate components to plant stress acclimation.

Balancing Growth and Defense: Role of TOR and SnRK1 Kinases in Biotic Stress and Cell Wall Damage Responses in *Arabidopsis*

Antea Mariani, *Simone Ferrari*

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Plants must dynamically coordinate growth and defense during biotic stress, reallocating metabolic resources from development toward immune functions. This growth–defense trade-off is governed by complex signaling networks integrating environmental, metabolic, and immune cues. Two evolutionarily conserved kinases, Target of Rapamycin (TOR) and SNF1-Related Kinase 1 (SnRK1), act as central and antagonistic regulators of this balance: TOR promotes anabolic metabolism and growth under energy-rich conditions, whereas SnRK1 is activated under energy limitation to induce catabolic pathways and stress adaptation.

In *Arabidopsis thaliana*, immune activation represses TOR activity, while SnRK1 positively regulates defense-related gene expression and intersects with hormone-mediated immune pathways. In parallel, perturbation of cell wall integrity (CWI), caused by mechanical stress or pathogen-derived enzymes, triggers immune-like responses that restrict growth and reinforce defense, suggesting strong links between CWI signaling and canonical immunity. This project aims to elucidate how TOR and SnRK1 integrate immune and CWI-derived signals to regulate the growth–defense trade-off. Using pathogen- and damage-associated elicitors and pharmacological manipulation of TOR activity, we will monitor TOR and SnRK1 activity through phosphorylation of downstream targets and in vivo biosensors. Genetic lines with altered TOR or SnRK1 activity will be used to assess the impact on pattern-triggered immunity, including ROS production, MAP kinase activation, callose deposition, calcium signaling and defense gene expression.

In addition, TOR and SnRK1 function will be analyzed during CWI perturbation induced by cellulose biosynthesis inhibition and mutations affecting cell wall composition or CWI signaling. Proximity labeling–based interactome analyses will identify upstream regulators and downstream effectors of TOR and SnRK1 under biotic stress and CWI perturbation. Integration of this data will enable the reconstruction of signaling networks underlying metabolic and developmental reprogramming.

Overall, this work will provide a mechanistic framework linking energy signaling, immune perception and cell wall integrity in plant stress responses.

Seed morphophysiological heterogeneity in *Lathyrus sativus* accessions

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Lathyrus sativus, commonly known as grass pea, is an orphan legume crop of significant interest in the context of modern agricultural challenges and climate change due to its resilience and nutritional properties. Despite its potential for sustainable farming systems, this species remains underutilized and understudied, particularly regarding intra-specific variability in seed quality.
The aim of this study is to investigate the morphophysiological heterogeneity of various *Lathyrus sativus* accessions, focusing on the relationship between seed mass and germinative performance. To characterize the seed mass distribution, weight analysis was performed on 400 individual seeds per accession. Based on the resulting distribution profiles, three size classes were isolated: "small" (weight < 10th percentile), "large" (weight > 90th percentile), and "mixed" (unfractionated control). Preliminary data obtained from six seed lots belonging to three *L. sativus* varieties (Maleme, Sofades and Limnos), collected between 2022-2025, generally presented a Gaussian distribution. Only one seed lot from Sofades variety presented a non-Gaussian distribution. Interestingly, germination tests conducted on this specific accession revealed that small seeds possess a lower Mean Germination Time (MGT) and a higher germination percentage compared to large seeds, while mixed seeds showed intermediate values. Accordingly, higher ROS emission during imbibition was detected in small seeds, suggesting an accelerated metabolic activation. This finding is supported by imbibition profiles, which indicate that small seeds reach the log phase significantly earlier than large seeds.
These data may suggest that, in Lathyrus sativus, smaller seed size does not preclude low vigour; instead, it appears associated with faster emergence. Such evidence raises questions regarding the adaptive strategies and resource partitioning during maturation. These findings lay the groundwork for future molecular investigations. Subsequent research will employ metabolomics to identify the biochemical pathways and molecular markers responsible for this morphophysiological differentiation, aiming to optimize seed selection and management for this legume species.

Session 4: Emerging technologies

Spectral signatures potentially allow real-time, nondestructive identification of root species in the field

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Supporting sustainable agriculture requires a deeper understanding of below-ground interactions under diversified crop mixtures. Current tools do not allow differentiation of root species without destructive sampling. This makes the study of crop mixtures and their belowground interactions laborious, leading to a reduction in the scale of research. There is an urgent need for standardized, cost-effective methods for root phenotyping, particularly under field conditions where high variability and logistical difficulties are common. To leverage root phenotyping as a tool for root species differentiation, it is essential to consider both architectural root traits and physicochemical traits. The UV (10–400 nm), VIS (400– 750 nm), NIR (750–1000 nm), and SWIR (1000–2500 nm) spectral regions offer significant potential for encoding root traits because of their sensitivity to specific absorption features that can represent a species' identity Thus, RGB cameras used in image-based approaches could be extended to include the NIR and SWIR spectral regions, enabling the development of a multispectral tool for analysing species-specific root physicochemical traits such as fiber, nitrogen, and carbon content. The multispectral camera system is configured to capture images at fixed, preselected wavelengths of interest coupled with minirhizotrons installed in the field. Processing and analysing such a unique root data type with optimized deep learning and machine learning potentially lead to high-throughput root mixture analysis.

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dsRNA production in cyanobacteria and microalgae for biocontrol of invasive species *Popillia japonica*

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Invasive pests pose a major threat to agriculture, and their impact is expected to increase as global trade rises and climate change continues. Their management is largely based on chemical pesticides that have negative impacts. RNA interference (RNAi) is a sequence-specific mechanism in eukaryotes that regulates gene expression. Strategies based on RNAi are now emerging in crop protection as a very promising alternative to chemical pesticides. The technology is based on the use of double-stranded RNAs (dsRNAs) to silence essential genes of a selected pest.

Nevertheless, cost-effective production of dsRNAs is a limiting factor for this technology. Microalgae can produce low-cost dsRNA in high yields and accuracy. The dsRNA can either be purified or delivered directly by the application of the microorganism itself. The direct application is of particular interest because it avoids the cost of purification and keeps stable the dsRNAs that could otherwise be easily degraded in the exogenous environment.
In this work, RNAi was explored for the control of the Japanese beetle *Popillia japonica* (Coleoptera: Scarabaeidae), an invasive species, highly polyphagous, which causes severe damages to crops. Two target sequences were expressed in two different microalgae: the model eukaryotic green alga C. reinhardtii and the novel, fast-growing cyanobacterium, *Picosynechococcus* PCC 11901. The dsRNAs were successfully accumulated in both microalgae as double strains.

The production process was then validated in lab-scale bioreactors up to 10 liters. The resulting material was collected and used for lethality tests on larvae and adult insects and for defoliation tests on infested grapevine plants. The results were promising, with greater lethality than in untreated insects and improved preservation of vine leaves. Furthermore, analysis of *P. japonica* transcripts showed a decreased transcript levels in individuals fed with dsRNA-expressing microalgae, confirming the uptake of dsRNA by the host and their interfering activity.

Assessing an In Vivo Approach to Validate CRISPR-Cas9 RNA Guides in Durum Wheat

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CRISPR-Cas9 technology has become one of the most extensively used methods for the introduction of precise modifications into plant genomes. Although numerous in silico tools are available to assist in the design of effective guide RNAs, in vitro and in vivo validations remain the most reliable approaches to confirm guide efficiency (D'Orso et al., 2024). In particular, in vivo validation systems better account for the biological context in which the CRISPR-Cas9 system operates. Given the considerable time and resource investment required for plant genome editing, a robust strategy for the selection of effective guides is essential.

In this study, we are evaluating an in vivo approach to validate guide RNAs intended for genome editing in durum wheat using an heterologous hairy root system in tomato (*Solanum lycopersicum*). This system allows to test the guides in a real biological context, although it does not provide information on potential off-target effects. We selected seven guides from a previous study in durum wheat (Camerlengo et al., 2020), that led to multiple editing events targeting the CM3 and CM16 genes, encoding amylase-trypsin inhibitor (ATI) proteins.

This method involves the cloning of each guide along with its corresponding target gene sequence into a single plasmid construct using the hierarchical Golden Gate cloning strategy resulting in seven final level-2 constructs. Each construct is then used to transform tomato cv. 'Micro-Tom' explants via *Agrobacterium rhizogenes* to induce hairy root formation. Each hairy root is subsequently genotyped using Sanger sequencing, as each root is expected to represent an independent editing event.

By comparing the outcomes with the editing events previously observed in durum wheat plants, we aim to assess the performance of this validation method in providing insights into the occurrence, frequency and types of genome editing events. This study contributes to enhance CRISPR-Cas9 applications in durum wheat by refining the guide RNA selection process.

From *Arabidopsis* to crop-relative species: unrevealing the hidden world of long-distance Ca^{2+} signalling in plants

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Over the last decades, research on how plants perceive and respond to environmental stimuli has risen. To investigate these processes in vivo, both biological tools and imaging technologies have rapidly evolved. In particular, the use of genetically encoded fluorescent indicators (GEFIs) has revolutionized real time in vivo studies of second messenger dynamics in plants. This approach has already revealed crucial aspects of plant biology, mainly through the use of high-end specialized microscopy systems with the model species *Arabidopsis thaliana*.
Among the various second messengers and signalling molecules, calcium ions (Ca^{2+}) and glutamate (L-Glu) play central roles in plant signal transduction, activating a wide range of signalling cascades in response to biotic and abiotic stimuli. However, despite the power of current imaging approaches, little is known about long distance signalling in adult *Arabidopsis* plants or in other species, particularly crops and crop relatives.
To overcome these limitations, we pursued two complementary strategies: i) the use of *Nicotiana benthamiana*, a widely adopted crop-relative species, engineered to express different GEFIs for in vivo monitoring of Ca^{2+} and L-Glu dynamics; ii) the development of the Macro Plant Projective Imaging system (MAPPI), an orthogonal, large field of view fluorescent imaging platform.
Combining these approaches enabled us to investigate Ca^{2+} and L-Glu dynamics from seedling to adult developmental stages under near physiological conditions. Moreover, the orthogonal configuration of the imaging system opened the possibility to study not only leaf to leaf signalling but also shoot to root and root to shoot Ca^{2+} waves triggered by stresses such as wounding, burning, flooding, and salt exposure.
In conclusion, these integrated approaches establish a foundation for dissecting systemic signal propagation at the whole plant level, across different species, and under diverse stress conditions.

IoT Microclimate Monitoring: Single-Plant Microchambers for Precision Phenotyping

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The integration of low-cost IoT systems represents a fundamental pillar of precision phenotyping, enabling continuous monitoring of genotype–environment interactions with high metrological rigor. In this context, a Single-Plant Microchambers (SPM) system was developed, designed as an independent growth unit for detailed physiological analysis. The physical structure of each SPM consists of a functional plastic base and a transparent dome, a design that ensures microclimatic isolation while allowing rapid access for sensor maintenance and calibration.
The current system configuration adopts a logic based on the ESP32 microcontroller, which acts as a Remote Terminal Unit. Each SPM integrates a strategic sensor set, including a DHT11 module for temperature and relative humidity, a BH1750 sensor for light intensity (lux), and an AGS02MA sensor used as a proxy for the volatilome (TVOC). A ventilation system based on PWM-controlled fans allows regulation of the specific humidity of each SPM. An implementation of the current system concerns the automated irrigation module, which aims to ensure optimal water control by combining data from the HS1136 capacitive soil-moisture sensor with a gravimetric weighing system based on load cells and HX711 sensors.
At the computational level, the collected time series will be analyzed with Spatio-Temporal Graph Neural Network (ST-GNN) models, with the aim of capturing spatial and functional dependencies among microchambers and enabling surrogate models for complex physiological trait estimation. Future methodological developments are oriented toward the evolution of the SPM into a closed-box multi-analysis chamber, featuring controlled airflow and the integration of a Zero Air Generator (ZAG), enabling accurate analysis of gas exchanges (CO_2 and H_2O) via IRGA and volatilome characterization through PTR-TOF-MS. Redesigning the dome toward a flat, transparent upper section will enable the integration of advanced imaging techniques for long-term, real-time monitoring.
In perspective, the system targets a 360° phenotyping and monitoring chamber.

Cold plasma priming as a strategy to burst rice tolerance to water scarcity

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Climate change is increasing the frequency and severity of water scarcity occurrence, posing a serious risk to global crop yield and consequently to food security. Rice (*Oryza sativa L.*) is particularly sensitive to water limitation during germination and early seedling development, making urgent the identification of strategies to enhance drought tolerance in this species.

This study investigates cold plasma (CP) seed priming as a novel, chemical-free approach to improve rice performance under water deficit. CP treatment works by the generation of reactive species, which, interacting with the seed surface, can increase seed wettability, facilitating water uptake during germination. The effect of CP priming has been evaluated in two rice varieties, Baldo and Cerere, showing different responses to simulated drought conditions. CP pre-treated and untreated seeds were grown under control conditions and water scarcity induced by treatment with polyethylene glycol (PEG). Therefore, phenotypic parameters, indicative of germination efficiency, seedling growth and oxidative stress response, were monitored under control and PEG conditions also in combination with seed priming by exposing seeds to different levels of CP. Morphological and biochemical analyses showed that CP differentially modulates plant performance under water deficit depending by treatment intensity in a genotype-dependent mode.

These findings suggest that CP seed priming is a promising, environmentally friendly strategy to enhance drought resilience in rice. The results emphasize the importance of adapting these innovative agricultural technologies to different genotypes to maximize benefits under stress, providing a sustainable approach to maintaining crop productivity in a changing climate.

Mechanisms of plant stress adaptation and physiological regulation induced by olive by-product-derived elicitors

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Olive mill waste (OMW) is produced in substantial quantities during olive oil extraction, and its uncontrolled dispersion can disrupt ecosystems because of its complex and highly reactive organic composition. Intriguingly, these by-products constitute a potential source of bioactive compounds which function as natural elicitors of plant immunity (1–3). Despite their promise, industrial applications are limited due to unsustainability of chemical solvents and low technology readiness. This study is an integral part of the OLinWASTE Horizon Europe initiative(4), which aims to establish a zero-emission system for the management of olive mill waste, reduce soil pollution and optimise resource efficiency. The project involves the development of sustainable extraction and separation processes to recover biocompounds from olive mill wastes. The fractions were chemically characterised for their glycan–phenolic profiles. Physiological and molecular analyses revealed that selected fractions activate key hallmarks of plant innate immunity, including the induction of defence-related gene expression, the activation of mitogen-activated protein kinases (MAPKs), and the accumulation of hydrogen peroxide (H_2O_2). Importantly, these immune responses were accompanied by the maintenance of normal plant growth, indicating a priming effect rather than a growth-defence trade-off. Overall, these results demonstrate that OMW-derived bioactive fractions can modulate plant stress physiology by triggering immune signalling pathways associated with enhanced stress resilience. This approach provides a sustainable strategy for improving crop performance under environmental stress, while supporting circular agriculture and reducing dependency on chemical pesticides.

Evaluation of wood distillate and Spirulina biostimulants to increase nickel hyperaccumulator-based phytomining in serpentine soil

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Phytomining harnesses hyperaccumulator plants to sustainably extract and recover trace elements from ultramafic (serpentine) soils. However, this technology is often constrained by low biomass yields. Biostimulants such as wood distillate (WD) and Spirulina have demonstrated significant success in enhancing plant growth and yield in different food crops, but their capacity to optimize phytoextraction in hyperaccumulating species represents a novel and unexplored frontier. In this study, the single and combined effects of WD and Spirulina - added at different concentrations and application methods - were evaluated on the growth and nickel (Ni) accumulation capacity of the hyperaccumulator *Odontarrhena decipiens* grown in serpentine soil. Shoot biomass peaked under the 1% Spirulina dose, yielding a substantial 350% increase compared to non-treated plants. Despite this sharp growth resulted in a dilution effect that lowered shoot Ni concentrations, the total harvestable Ni was >200% higher in this treatment, indicating biomass production as the primary driving force behind the enhanced phytoextraction yield. On the other hand, the 0.3% dose of foliar sprayed WD increased shoot Ni concentration by 20% but had much lower effects on shoot biomass, leading to lower levels of harvestable Ni compared to Spirulina. Available soil Ni concentrations doubled in Spirulina treatments compared to non-treated plants, suggesting that increased Ni mobility in the soil could as well play a critical role in maximizing shoot Ni yields. These observations suggest divergent mechanisms of biostimulation for enhancing Ni phytomining with hyperaccumulators and highlight that both WD and Spirulina can be valorized and incorporated into phytomining strategies.

Expansion of the plant CRISPR-Cas platform: evaluation of the genome editing efficiency of the CoCas9 nuclease in *Solanum lycopersicum* hairy roots

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What makes the CRISPR/Cas system particularly powerful is its ability to introduce precise targeted mutations in genomic loci. However, this potential is often limited by several factors, among the most significant being the constraints of the protospacer adjacent motif (PAM). To overcome such limitations and broaden the application spectrum of plant gene editing, the exploration across the rich repertoire of Cas9 variants emerging from microbial biodiversity to identify novel tools with expanded targeting capabilities has become a key research activity.
Here, we report the first evaluation of the genome editing performance of CoCas9 in plants, a novel compact Cas9 ortholog isolated from human microbiome and characterised by recognition of a degenerated PAM motif. As a proof of concept, we built several constructs using a plant-codon-optimised version of CoCas9 to target tomato genes exploiting the versatile hairy roots experimental system: DWARF gene, involved in brassinosteroids biosynthesis; HQT gene, essential for chlorogenic acid biosynthesis; and Woolly gene, responsible for trichome formation.
The results obtained, clearly demonstrate that CoCas9 is functional in plants and capable of efficiently inducing several kinds of editing events, including large deletions and small indels. Nevertheless, its activity is highly dependent on sgRNA sequence.
In addition, the use of tomato hairy roots proved particularly advantageous, allowing the rapid recovery of multiple independent transformation events with extremely high efficiency and enabling the characterisation of mutation effects on HQT gene also at the metabolic level.
To better define the efficiency of the CRISPR/CoCas9 system in direct comparison with the widely used SpCas9 an NGS-based analytical approach is currently being employed to accurately evaluate the nuclease performance, in terms of both conventional and Base Editing across multiple common targets.
The adoption of CoCas9 may represent a crucial step forward in enriching the plant genome editing toolbox, thereby enhancing plant biotechnology research.

Plasma priming as a tool for modulating germination, chemical and transcriptional profiling in *Cannabis sativa* seeds

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Plasma priming is a novel seed enhancement technique that uses non-thermal plasma to modify the seed surface and trigger physiological and molecular responses. Plasma is created by ionizing gas molecules exposed to a radiofrequency electromagnetic field. Hence, plasma is composed of reactive species that can modify the seed surface, thereby improving hydrophilicity and water uptake. Although these treatments are rapid, their efficacy depends on optimization of several parameters, such as gas type and treatment time. The present study aimed to develop and optimize plasma priming for *Cannabis sativa* (hemp) seeds by evaluating the effects of different gas phases, pressure, and exposure time. Hemp cultivation is gaining increasing interest driven by its applications in the agrifood, textile, and pharmaceutical fields. Two seed lots of a commercial hemp variety with distinct seed quality levels were used. The physiological effects on seed germination were determined by measuring several germination and developmental indices. Additional analyses included the evaluation of wettability levels, surface composition and structure by X-ray photoelectron spectroscopy (XPS), water uptake, oxidative status, and selected gene expression profiles. Integrative data analyses showed that oxygen-based plasma treatments applied for short exposure times improved germination, likely due to enhanced hydrophilicity. Based on the gene expression data, we hypothesize that this treatment acted as a moderate stress, inducing the seed pre-germinative metabolism and cell cycle regulation during imbibition. However, prolonged exposure to oxygen plasma resulted in detrimental effects, possibly attributed to the excessive water uptake and ROS overproduction. In summary, when properly optimized, plasma priming represents a transformative, environmentally sustainable agricultural technology that enhances seed germination by using ionized gas to alter seed surface properties and activate metabolic defence pathways, thereby providing a chemical-free alternative to conventional treatments.

Seed biopriming in frames: understanding and improving its effectiveness through image-based analyses

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Seed biopriming, one of the sustainable methods to improve germination and early seedling establishment can be achieved by applying bacterial spores from beneficial microorganisms prior to sowing. However, the efficient use of this technique is challenged by the limited understanding of how spores adhere to the seed at a surface level and by the lack of non-destructive methods useful to differentiate treated and nontreated seeds, as well as to assess the optimal spore doses. In this study, a novel framework that utilizes imaging analysis, integrating advanced microscopy technique (scanning electron microscopy, SEM) and muti-spectral imaging (MSI), was conducted on seeds of orphan legumes treated with *Bacillus subtilis* spores. Species such as Lathyrus sativus (grass pea), *Pisum sativum* var. arvense (forage pea), and Trigonella foenum-graecum (fenugreek) were used, including two varieties per species. This pilot study aims to understand the impact of surface topography and roughness on spore adhesion and to assess the efficacy of MSI in detecting the presence of non-pathogenic bacteria such as B. subtilis on the seed surface. Among the six varieties investigated, the grass pea Maleme-107 and Sofades seeds showed contrastive surface profiles and were selected for MSI evaluation. Analysis of the mean reflectance percentage across 20 spectra enabled detection of spore presence at 0h and 6h after imbibition, specifically at wavelengths of 645, 660, and 690 nm. This has provided a range potentially effective for discriminating bioprimed from untreated seeds. A genotype-dependent effect was also observed. These preliminary results highlight the potential of the integrated use of MSI and SEM tools to better understand the dynamics of the spore-seed interactions and to enhance the overall value of biopriming protocols applied to orphan legumes and other crop species.

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