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SOCIETA' ITALIANA di
BIOLOGIA VEGETALE

XIV CONGRESS OF THE ITALIAN SOCIETY OF PLANT BIOLOGY

16-19 September 2025

University of Palermo, Italy
Viale Delle Scienze - Palermo
Building n.19

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DIPARTIMENTO DI SCIENZE E TECNOLOGIE BIOLOGICHE CHIMICHE E FARMACEUTICHE (STEBICEF)

Consiglio Nazionale delle Ricerche

XIV National Congress
of the Italian Society of Plant Biology

University of Palermo
16-19 September 2025

PROGRAMME

16 September 2025

12:30-14:00 Registration and poster installation

14:00-14:30 **Opening Ceremony**

Fiorella Lo Schiavo, President of the Italian Society of Plant Biology

Massimo Midiri, Rector of the University of Palermo

Rosario Schicchi, Director of the Botanical Garden of Palermo

Symposium 1

PLANT HORMONES: FROM SIGNALING TO DEVELOPMENT

Chairs: **Fiorella Lo Schiavo**, University of Padova,

Elide Formentin, University of Padova

14:30-15:10 **JIŘÍ FRIML**

Institute of Science and Technology Austria (ISTA) Austria

Auxin Signaling Revisited: A Fresh Perspective on Second Messengers in Plants

15:10-15:40 **RAFFAELE DELLO IOIO**

Dept. Biology and Biotechnology *C. Darwin* Sapienza University of Roma

Diverse Control of Asymmetric Cell Division Shapes Root Patterning Across species.

15:40-15:55 **Elisa Maricchiolo***,* University of Urbino Carlo Bo

Intracellular ERAD-mediated regulation of CLAVATA3: the involvement of a novel maturation mechanism controlling plant meristem differentiation

15:55-16:10 **Elena Salvi**, University of Pisa

Hormones as patterning agents and drivers of change in plant development: the tale of auxin in petal morphogenesis

16:10-16:25 **Marta Del Bianco,** Italian Space Agency of Roma

Angle dependence as a unifying feature of root graviresponse modules

16:25-17:00 **COFFEE BREAK**

17:00-17:15 **Eva Mari Gomez Alvarez,** Institute of Plant Sciences of Pisa

The impact of rainfall regimes on barley seed development underlies genomic variation for germination after flooding

17:15-17:30 **Barbara Molesini,** University of Verona

Molecular dissection of the role of Aucsia-1 and Aucsia-2 in tomato root development

17:30-17:45 **Diana Gervasoni**, CNR - Institute of Agricultural Biology and Biotechnology of Milano

CRISPR-mediated knockout of SlMYB60 highlights functional divergence from its Arabidopsis ortholog

17:45-19:00 **Round Table on Scientific Communication**

Chair: **Manuela Rigano,** University of Napoli Federico II

Ruggero Rollini, Department of Chemistry, University of Bologna

Giacomo Moro Mauretto, Entropy for Life

19:00-20:30 **WELCOME COCKTAIL PARTY**

17 September 2025

Symposium 2

CUTTING EDGE APPROACHES IN PLANT BIOLOGY

Chairs: **Roberto De Michele,** CNR-IBBR of Palermo
Stefano D'Alessandro, University of Torino

09:00-09:40 **ALEXANDER JONES**

Sainsbury Laboratory Cambridge University

Revealing Cellular Decision Making for Plant Plasticity

09:40-10:10 **ALEX COSTA**

Dept. of Biosciences University of Milano

Plant Calcium Signaling: From the Microscope Slide to the Real World

10:10-10:25 **Lucrezia Luciani,** CNR - Institute of Agricultural Biology and Biotechnology of Milano

Function of the Arabidopsis pBiP3 promoter under different stress conditions

10:25-10:40 **Maria Teresa Chiofalo,** University of Messina

Seeing the unseen: microplastics effects on tomato hydraulics and non-structural carbohydrates

10:40-10:55 **Francesco Fioriti,** Sant'Anna School of Advanced Studies, Pisa

Chemical genetics approach reveals new molecules as activators of plant hypoxic response

10:55-11:30 **COFFEE BREAK**

11:30-11:45 **Alessandra Boccaccini,** Campus Bio-Medico University of Roma

ATAC-seq-based profiling of cis-regulatory elements in Arabidopsis thaliana seedlings

11:45-12:00 **Lucien Davide Patono,** University of Torino

Multi-scale assessment of grapevine drought priming and anisohydric responses induced by the biostimulant YaraAmplix OPTIVI

12:00-12:15 **Cosenza Francesco,** IBBR-Institute of Biosciences and Bioresources of Palermo

Developing Ammonium Transporter Sensors specific to mycorrhizae in Lotus japonicus and Glycine max

12:15-13:30 **POSTER SESSION A**

13:30-14:30 **LUNCH**

Symposium 3

EVOLUTIONARY AND FUNCTIONAL BIOCHEMISTRY IN PLANTS

Chairs: **Alessandro Alboresi, University of Padova**

Mirko Zaffagnini, University of Bologna

14:30-15:10 **ALISTAIR McCORMICK**

Institute of Molecular Plant Sciences School of Biological Sciences

University of Edinburgh

Expanding The Scope of Pyrenoid-Based CO_2-Concentrating Mechanisms To Enhance Plant Photosynthesis

15:10-15:40 **ROBERTO BASSI**

Stazione Zoologica Anton Dohrn, Napoli

From Desert to Seabed: Nature-Based Engineering of Photosynthetic Light Harvesting Systems

15:40-15:55 **Alessandra Norici,** Polytechnic University of Marche, Ancona

Functional response of diatoms to a changing ocean chemistry

15:55-16:10 **Maria Meloni,** University of Bologna

Deciphering the redox regulation of the Calvin-Benson cycle enzyme fructose-1,6-bisphosphatase from Chlamydomonas reinhardtii

16:10-16:25 **Sara Natale,** University of Padova

Exploring photosynthetic diversity: the role of phylogeny and environmental plasticity

16:25-17:00 **COFFEE BREAK**

17:00-17:15 **Luca Tadini,** University of Milano

Protein homeostasis and chloroplast quality control: mis-localization as a signaling mechanism

17:15-17:30 **Paolo Korwin Krukowski,** University of Torino

Evolutionary insights into β-cyclocitral signaling in Physcomitrium patens

17:30-17:45 **Beatrice Giuntoli,** University of Pisa

Hydrogen sulfide modulates plant responses to hypoxia via modification of the oxygen-sensing Plant cysteine oxidase (PCO) enzymes

17:45-18:00 **RASI CALDOGNO AWARD lecture**

Matteo Pivato- University of Verona

The molecular basis of Chlamydomonas reinhardtii responses to the environment: the role of intracellular Ca2+ signalling and minor antenna proteins

18:00-20:00 **General Assembly of the Italian Society of Plant Biology**

18 September 2025

Symposium 4

PLANT-ENVIRONMENT INTERACTIONS

Chairs: **Michela Zottini,** University of Padova

Maria Concetta de Pinto, University of Bari

09:00-09:40 **PHILIPPE GALLUSCI**

UMR EGFV, Bordeaux University, Department of environmental sciences, INRAE, Bordeaux Science Agro,Epigenetic memories in plants

09:40-10:10 **FRANCESCO LORETO**

Dept. of Biology, University of Napoli Federico II

Deciphering Plant Interactions with Friends and Foes: the VOC Languages

10:10-10:25 **Stefano D'Alessandro,** University of Torino

Anterograde regulation of photosynthetic activity by the TOR kinase in plants

10:25-10:40 **Diego Losada Dìaz,** University of Padova

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

10:40-10:55 **Guido Domingo,** University of Insubria

The root code cracked: deciphering the complex interplay between root exudation, drought and microbiota in wheat

10:55-11:30 **COFFEE BREAK**

11:30-11:45 **Ginevra Maria Eloise Peppi,** University of Bologna

Mechanistic and redox-regulatory insights into plant aldo-keto reductase-mediated GSNO catabolism

11:45-12:00 **Chiara Pedalino,** University **of** Roma Tre

Auto-inhibited Ca^{2+}-ATPases ACA8 and ACA10 mediate wound-induced long-distance Ca^{2+} waves and ROS-dependent guard cell responsiveness in Arabidopsis

12:00-12:15 **Maria Manuela Rigano,** University of Napoli Federico II

Investigating shade tolerance in a S. pennellii introgression line

12:15-12:30 **Nadia Bazihizina,** University of Firenze

Ultraviolet B radiations improve salt-induced responses in the facultative halophyte Chenopodium quinoa

12:30-13:30 **POSTER SESSION B**

13:30-14:30 **LUNCH**

Symposium 5

PLANT BIOTIC INTERACTIONS

Chairs: **Simone Ferrari**, University of Roma La Sapienza

Raffaella Balestrini, CNR-IBBR of Bari

14:30-15:10 **SIMON STAEL**

Dept. of Molecular Sciences, The Swedish University of Agricultural Sciences Uppsala

Enhancing Pesticide Specificity Through Insights into Plant Wound Responses

15:10-15:40 **BENEDETTA MATTEI**

Dept. of life, Health, and Environmental Sciences, University of L'Aquila

Enzymatic Oxidation of Cell Wall Oligosaccharides: Implications for Plant Defenses and Cell Wall Remodeling

15:40-15:55 **Sarah Giulietti,** University of Roma

Deciphering HPCA1's role in OG-mediated defense and pathogen resistance

15:55-16:10 **Emma Olmi,** Sant'Anna School of Advanced Studies, Pisa

Submergence primes Arabidopsis thaliana for enhanced resistance to Botrytis cinerea

16:10-16:25 **Alessandro Cavazzani,** University of Roma Tor Vergata

14-3-3ε acts as a negative regulator of the response of Arabidopsis thaliana to Pst DC3000

16:25-16:40 **Ambra Selene Parmagnani,** University of Milano

Endophytes allies against climate change: modulation of growth and ABA signaling in arabidopsis thaliana under drought stress

16:40-16:55 **Sabella Erika,** University of Salento

Xylem anatomical changes as adaptative response to long term Xylella fastidiosa infection in the susceptible olive cultivar Cellina di Nardò

16:55-17:30 **COFFEE BREAK**

18:00-**Guided Tour of the Botanical Garden**

20:30-**Social Dinner**

19 September 2025

Symposium 6 - *organized by SIBV Junior Members*

NEW FRONTIERS IN GREEN BIOTECHNOLOGIES

Chairs: **Ambra Parmagnani,** University of Milano

Edoardo Tosato, University of Torino

09:00-09:40 **PATRICIA LOPEZ-CALCAGNO**

School of Natural and Environmental Science, Newcastle University

Using Transgenics for Food Security, Challenges and Insights

09:40-10:10 **GIORGIO PERIN**

Dept. of Biology, University of Padova

Investigation of the win-win cooperation between C and N assimilation for biotechnological applications

10:10-10:25 **Davide Canini,** University of Verona

Genetic engineering of Nannochloropsis oceanica to produce canthaxanthin and ketocarotenoids

10:25-10:40 **C**larissa **Zanotti**, University of Roma Tor Vergata

Anion exchange chromatography-based purification of exosomes from Brassica oleracea L.: Molecular Profiling And Bioactivity In Human Cells.

10:40-10:55 **Valentina Scafati,** University of L'Aquila

Exploiting the reducing power of cell wall oligosaccharides for the enzymatic synthesis of novel compounds

10:55-11:10 **Valeria Villanova,** University of Palermo

Antioxidant potential of halophilic microalgae from salt ponds of Trapani

11:10-11:45 **COFFEE BREAK**

11:45-12:30 **Round Table: Beyond the Lab: boost your research impact**

Chairs: **Sara Natale**, University of Padova

Noemi Gatti, University of Torino

Alessandra Boccaccini, Campus Bio-Medico University of Roma

Giorgio Perin - Dept. of Biology, University of Padova - *From the lab to market: making your research profitable*

Vincenzo Lionetti - University Roma, La Sapienza - *Coordinating the European initiative OLinWASTE: insights and lessons learned as project leader*

Patricia Lopez-Calcagno - School of Natural and Environmental Science, Newcastle University - *Empowering scientific results on a global scale: the RIPE consortium*

12:30-13:00 **CONGRESS CLOSURE**

Symposium 6 *New Frontiers in Green Biotechnologies* has been organized by the following SIBV Junior Member:

Alessandra Boccaccini – Campus Bio-Medico University of Roma

Pasquale Creanza – University of Urbino

Noemi Gatti – University of Torino

Libero Gurrieri – University of Bologna

Thais Huarancca Reyes – University of Pisa

Elisa Maricchiolo – University of Urbino

Cristina Morabito– University of Padova

Sara Natale – University of Padova

Ambra Selene Parmagnani – University of Milano

Edoardo Tosato – University of Torino

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Oral Presentations

01 PLANT HORMONES: FROM SIGNALING TO DEVELOPMENT

Auxin signaling revisited: new roles for second messengers

JIŘÍ FRIML[*], Huihuang Chen, Linlin Qi, Minxia Zou

Institute of Science and Technology Austria (ISTA), Klosterneuburg, Austria

Keywords: Phytohormones, Auxin, ubiquitination, cAMP, cGMP

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

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

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

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

Chen H, Qi L, Zou M, Lu M, Kwiatkowski M, Pei Y, Jaworski K, Friml J. (2025) TIR1-produced cAMP as a second messenger in transcriptional auxin signalling. Nature, Mar 5. doi: 10.1038/s41586-025-08669-w

Qi L, Kwiatkowski M, Chen H, Hoermayer L, Sinclair S, Zou M, Del Genio CI, Kubeš MF, Napier R, Jaworski K, Friml J. (2022) Adenylate cyclase activity of TIR1/AFB auxin receptors in plants. Nature 611(7934):133-138. doi: 10.1038/s41586-022-

A conserved genetic program patterns root ground tissue in angiosperms

DELLO IOIO RAFFAELE, Bertolotti G.[1], De Vivo M. [1], Kirschner G.[2], Scintu D. [1], Simon R. [2], Sabatini S. [1],

(1)Dipartimento di Biologia e Biotecnologie “Charles Darwin” Università di Roma Sapienza
(2)Institute of Developmental Genetics Heinrich Heine University Düsseldorf

One of the fundamental questions in biology is understanding the molecular mechanisms behind interspecific morphological diversity. In plants, roots exhibit striking variations in phenotype across species. A classic example of such diversity is the number of cortical layers, which can range from a single layer to multiple layers. Comparative studies of the model plant Arabidopsis thaliana (which has only one cortical layer) and its close relative Cardamine hirsuta (which possesses two cortical layers) have provided insights into the molecular mechanisms that regulate cortical layer formation. Specifically, we have shown that the Cortex and Endodermis Mix (CEM) tissue, absent in A. thaliana, undergoes asymmetric division to form the second cortical layer and the endodermis in C. hirsuta. This division is triggered by the activity of HOMEODOMAIN LEUCINE ZIPPER III (HD-ZIPIII) transcription factor PHABULOSA (PHB) in the CEM domain of C. hirsuta. In contrast, in A. thaliana, PHB activity is confined to the vasculature due to regulation by miR165/6. Here, we demonstrate that PHB controls the number of cortical layers by promoting the activity of the plant hormone cytokinin in the CEM cells. We show that PHB coordinates with the transcription factor SHORT ROOT (SHR) to ensure a robust expression of the cell cycle regulator CYCLIND6;1 (CYCD6;1), a key process essential for the proper formation of the double cortical layer in C. hirsuta. This pathway, essential for the formation of the double cortical layer in C. hirsuta, is conserved across angiosperms with multiple cortical layers. Our findings reveal an elegant molecular circuit through which cortical patterning diversity originates among plant species.

Intracellular ERAD-mediated regulation of CLAVATA3: the involvement of a novel maturation mechanism controlling plant meristem differentiation

Elisa Maricchiolo ***(1)*** *- Pasquale Creanza (1) - Michela Osnato (1) - Sara Scataglini (1) - Francesca De Marchis (2) - Michele Bellucci (2) - Enrico Magnani (3) - Miryam Iannaccone (3) - Sandrine Choinard (3) - Wenjia Xu (3) - Federico Perozeni (4) - Matteo Ballottari (4) - Andrea Pompa (1)*

(1) Università degli studi di Urbino Carlo Bo, Dipartimento di scienze biomolecolari, Urbino, Italia - (2) CNR, Istituto di Bioscienze e Biorisorse, Perugia, Italia - (3) INRA Versailles Grignon, Institut Jean-Pierre Bourgin, INRAE, AgroParisTech, CNRS, University of Paris-Saclay, Versailles, Francia - (4) Università degli studi di Verona, Dipartimento di biotecnologie, Verona, Italia

The indefinite growth capacity and organogenesis in plants are guaranteed by meristems, tissues where cells are in continuous division and differentiation, regulated by intercellular signals. In the Shoot Apical Meristem (SAM) of *Arabidopsis thaliana*, CLAVATA3 (CLV3) proteolytic form establishes a negative-feedback loop modulating the expression of the stem cell-promoting transcription factor WUSCHEL (WUS)[1], thus mantaining SAM functions throughout the plant's life, balancing the loss of daughter cells during organogenesis and stem cell maintenance. Previous studies suggest that CLV3 is processed by apoplastic proteases into its active dodecapeptide form. However, recent findings in *Nicotiana tabacum* propose an alternative intracellular mechanism of maturation, via the Endoplasmic Reticulum-Associated Degradation (ERAD) pathway, naturally used by cells to degrade misfolded proteins[2]. To shed light on this process, CLV3-GFP was expressed in *A. thaliana* and *N. tabacum* plants and protoplasts, and its localisation was monitored with biomolecular and imaging approaches. The possible role of ubiquitination, a key initiator of proteasome-mediated degradation, was also investigated. Moreover, a transgenic line of *Chlamydomonas reinhardtii* overexpressing *CLV3-YFP* was developed to study CLV3 processing in an organism lacking meristems and endogenous CLV3. Preliminary data suggest a maturation pathway distinct from canonical secretion, varying across different biological systems.

References:

[1] Brand, U., Fletcher, J. C., Hobe, M., Meyerowitz, E. M., & Simon, R. (2000). Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by CLV3 activity. *Science*, *289*(5479), 617–619.

[2] De Marchis, F., Colanero, S., Klein, E. M., Mainieri, D., Prota, V. M., Bellucci, M., Pagliuca, G., Zironi, E., Gazzotti, T., Vitale, A., & Pompa, A. (2018). Expression of CLAVATA3 fusions indicates rapid intracellular processing and a role of ERAD. *Plant Science*, *271*, 67–80.

Hormones as patterning agents and drivers of change in plant development: the tale of auxin in petal morphogenesis

Elena Salvi **[(1)]** *- Stefano Gatti (2) - May Yeo (2) - Riccardo Di Mambro (1) - Edwige Moyroud (2)*

(1) Department of Biology, University of Pisa, Pisa, Italia - (2) Sainsbury Laboratory, Univeristy of Cambridge, Cambridge, Regno Unito

In multicellular organisms, morphogenesis relies on patterning molecules. Phytohormones evolved as mobile regulators coordinating environmental responses and plant development, allowing morphogenetic programmes and tropisms for organ patterns and shapes.Angiosperm petals and their patterns serve as an intriguing system to investigate these processes, given their accessibility, robustness to environmental fluctuations and their role in plant fitness by protecting reproductive organs and attracting pollinators. While some genetic regulators have been uncovered, the role of hormones in petal cell specification is elusive.

Hibiscus trionum petals display a “bullseye” pattern, made of an iridescent purple proximal region, a velvety white distal region and a narrow boundary in between. Using transcriptomics, we identified auxin-related genes differentially expressed (proximo-distally) across the petal. To explore their function, we generated *H.trionum* reporter, overexpressor and mutant lines which reveal a differential auxin response across developing petals, influencing petal growth and bullseye size, but not bullseye initiation. Since pollinators recognize differences in bullseye sizes and their interaction with flowers is influenced by corolla shapes, these results have ecological and evolutionary implications. Our findings suggest that auxin-mediated mechanisms contribute to petal morphogenesis and can generate variations in petal patterns and shapes.

Moreover, our results exemplify how using petals as a model will serve future investigations to decipher how hormonal crosstalks and regulations of development underlie potentially the emergence of plant diversity through petal shape determination and pollinator-based reproductive isolation.

Angle dependence as a unifying feature of root graviresponse modules

Marta Del Bianco (1) *- Suruchi Roychoudhry* (2) *- Katelyn Sageman-Furnas* (3) *- Harry Taylor* (2) *- Iftekhar Showpnil* (4) *- Chris Wolverton* (4) *- Jiri Friml* (5) *- Stefan Kepinski* (2)

(1) *Italian Space Agency, Italian Space Agency, Rome, Italia* - (2) *University of Leeds, University of Leeds, Leeds, Regno Unito* - (3) *Duke University, Duke University, Durham, Stati Uniti D' America* - (4) *Ohio Wesleyan University, Ohio Wesleyan University, Delaware, Stati Uniti D' America* - (5) *Institute of Science and Technology Austria, Institute of Science and Technology Austria, Klosterneuberg, Austria*

Gravitropism, the patterning of post-embryonic growth in relation to the gravity vector, allows plants to optimise the use of limited and non-homogenous resources in their immediate environment. Since the current model of root gravitropism has not been able to integrate all aspects of the response (perception, response, and behaviour), research on gravitropism has been dominated by different theories attempting to conceptualise each aspect individually.

In this work, we sought to reevaluate all the main components of the root graviresponse through the lens of angle-dependence. We show angle-dependence in Cholodny-Went-based auxin asymmetry and growth response, which we tracked back to angle-dependent variation in PIN asymmetry and statolith sedimentation in the columella. Thanks to this approach, we were also able to show that different PINs and columella cell tiers play distinct roles in establishing the asymmetric auxin gradient at different angles and postulate the importance of the vertical flux through the columella. Our findings provide a fundamental framework to further explore the mechanisms that regulate angle-dependent gravitropic response, with major implications for engineering root architecture in crop plants.

02 CUTTING EDGE APPROACHES IN PLANT BIOLOGY

Revealing Cellular Decision Making for Plant Plasticity

Alexander Jones

Sainsbury Laboratory Cambridge University

Plants, despite lacking sensory organs and centralized information processing, thrive in dynamic and challenging environments. The plant hormones play a key role in plants' ability to respond to the environment, for example abscisic acid (ABA) in water stress and salicylic acid (SA) in immunity stress. We have developed FRET biosensors for four plant hormones that are high-resolution and genetically-encoded, enabling us to visualize single-cell hormone dynamics in living plants. This has allowed us to uncover how hormonal signals are redistributed by environmental cues and the quantitative functions of these dynamics. Using ABA CONCENTRATION AND UPTAKE SENSOR 2 (ABACUS2) we discovered cellular ABA dynamics that coordinate root growth with leaf humidity stress, offering insights for irrigation agriculture under climate change. More recently, we engineered SALICYLIC ACID SENSOR 1 (SALICS1) by co-opting SA-mediated receptor protein interactions. Following optimisation along with in vitro and in vivo validations, we deployed SALICS1 to track surges of SA hormone spreading in response to a series of less friendly biotic interactions, i.e. viral, bacterial, and fungal pathogens. A future goal is to use these and other tools not only to reveal how plants respond to environment challenges, but also to engineer precise improvements to hormone dynamics to help crops thrive under stress.

Plant Calcium Signaling: From the Microscope Slide to the Real World

Alex Costa
Dipartimento di Bioscienze, Università degli Studi di Milano

Calcium (Ca^{2+}) signaling is a central regulator of plant physiology, coordinating responses to development and stress across cellular, tissue, and whole-plant scales. In Arabidopsis, glutamate receptor-like channels (GLRs) play a pivotal role in systemic Ca2+ signaling, linking environmental stimuli to jasmonic acid (JA)-dependent stress adaptation. Among these, AtGLR3.3 is essential for propagating long-distance Ca2+ waves triggered by leaf injury or osmotic stress.

This seminar will first explore how in vivo activation of AtGLR3.3 depends on its ligand-binding domain, which senses L-glutamate that accumulates in the leaf apoplast upon mechanical wounding, burns, or hypo-osmotic stress. Combining live imaging and genetics, we demonstrate that apoplastic L-glutamate accumulation precedes AtGLR3.3 activation, driving cytosolic Ca2+ elevations and downstream JA signaling.

While Arabidopsis has been instrumental in uncovering these mechanisms, translating such research to crops presents a major technological challenge: conventional imaging systems cannot accommodate larger plants. To overcome this limitation, we developed the MAcro Plant Projection Imaging (MAPPI) platform, an affordable, modular, dual-view system optimized for soil-grown plants like Nicotiana benthamiana. Using MAPPI, we captured systemic Ca2+ and L-glutamate dynamics in real time, revealing conserved long-distance signaling mechanisms previously uncharacterized outside Arabidopsis.

By integrating mechanistic insights from model systems with scalable imaging tools, this work bridges the gap between fundamental discovery and possible agricultural application, offering new avenues to engineer stress resilience in crops.

Developing Ammonium Transporter Sensors specific to mycorrhizae in Lotus japonicus and Glycine max

Francesco Cosenza [(1)] - *Roberto De Michele* [(1)]

[(1)] *Institute of Biosciences and Bioresources (IBBR), Italian National Research Council (CNR), Palermo, Italia*

Arbuscular mycorrhizal (AM) symbiosis enhances plant nitrogen acquisition by promoting the uptake of multiple nitrogen forms, including ammonium. However, the regulation of plants AM-inducible ammonium transporters (AMTs) remains largely unexplored. Thus, the development of tools able to monitor AMT dynamics is crucial to obtain a better understanding of plant ammonium uptake mechanisms. To evaluate AMT activity, we developed fluorescent ratiometric sensors for *LjAMT2;2*, from *Lotus japonicus*, and *GmAMT4;1*, from soybean (*Glycine max*), generating multiple constructs with two types of ratiometric fluorophores, GO-Matryoshka and GA-Matryoshka. Fluorophores were inserted into the intracellular loop connecting the transmembrane helices V and VI of the proteins encoded by *LjAMT2;2* and *GmAMT4;1*. Multiple insertion sites were explored to identify the optimal sensor configuration. Additionally, constructs incorporating a 15-nucleotide linker sequence (GRAIA), derived from the *Arabidopsis thaliana* homolog of the AMTs, were designed to test sensor performance. Constructs were then transformed into a yeast strain deficient in endogenous ammonium transporters. While initial growth on ammonium was not observed, functional mutants emerged after prolonged selection. Current work is directed toward quantifying transporter-mediated ammonium uptake. The application of these sensors in plants could allow a better understanding of ammonium uptake and its regulation under symbiotic conditions.

ATAC-seq-based profiling of cis-regulatory elements in Arabidopsis thaliana seedlings

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Plants live in ever-changing environments and must constantly adjust their growth to survive. This plasticity is mediated by the regulation of gene expression, driven by intricate interactions between transcription factors (TFs) and the chromatin. The DNA elements bound by TFs are typically located within regions of open chromatin, known as cis-regulatory elements (CREs), which are emerging as key players in trait variation, crop domestication, and species adaptation. Using the ATAC-seq (Assay for Transposase-Accessible Chromatin) approach, we identified open chromatin regions in ***Arabidopsis thaliana*** seedlings involved in light-mediated responses. By integrating chromatin accessibility data with TF binding profiles and gene expression analysis, we reconstructed a regulatory network underlying seedling responses to light fluctuation. In particular, we found that open chromatin conformation facilitates access of TFs to their binding motifs, rapidly triggering gene expression upon shade perception, which is primarily driven by a transient increase in TFs stability and DNA occupancy.

This approach can be extended to a wide range of environmental stimuli, enabling functional correlations between CRE activity and gene expression dynamics. Lastly, recent advances in nuclear purification and manipulation will allow the application of this strategy at tissue- and organ-specific resolution, offering new insights into the spatial and temporal dynamics of plant environmental responses.

Function of the Arabidopsis pBiP3 promoter under different stress conditions

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Adverse environmental conditions can cause accumulation of misfolded proteins in the endoplasmic reticulum (ER), triggering ER stress and activating the unfolded protein response (UPR), a signalling pathway that restores ER homeostasis by enhancing the production of folding chaperones, reducing protein synthesis, and degrading misfolded proteins. The key UPR sensor is BiP, an endoplasmic reticulum (ER)-resident HSP70 protein that promotes the folding of all secretory proteins, including seed storage proteins. In Arabidopsis, BiP1 and BiP2 are constitutive, while BiP3 is inducible by stress only. The BiP3 promoter (pBiP3) has potential bidirectional activity, upstream and downstream, on *BiP3* and *RBOHB* (oxidative stress marker). To investigate on this potential dual stress-related function, we have produced a stress sentinel model plant by transforming Arabidopsis Col-0 with a pBiP3 promoter activity construct in which the *EGFP* and *mCherry* sequences replaced *BiP3* and *RBOHB*, respectively. We also produced double transgenic lines, in which the above-described construct was inserted into plants constitutively expressing either 27kDa gamma zein or 16kDa gamma zein, two maize prolamins that trigger the UPR at different levels. Sentinel plants were exposed to various abiotic stresses (salt, chemicals, hypoxia). By analyzing tissue-specific fluorescence, we mapped the spatial expression of ER and oxidative stress markers, providing information on the activity of pBiP3. This model also offers a tool for real-time, non-invasive detection of environmental stresses in plant.

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Multi-Scale Assessment of Grapevine Drought Priming and Anisohydric Responses Induced by the Biostimulant YaraAmplix OPTIVI

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We tested the biostimulant (BS) YaraAmplix OPTIVI for improving drought tolerance in *Vitis vinifera* cv. Merlot through a pot experiment conducted in a high-throughput phenotyping greenhouse. BS was applied as a priming treatment before drought stress, which progressed from well-watered (WW) to moderate and severe water deficit, followed by a 10-day recovery phase. Physiological, metabolic and transcriptomic analyses were performed at key timepoints.

BS-treated plants developed thicker, denser leaves and showed reduced stem elongation. Under WW conditions, transpiration (E) decreased without affecting carbon assimilation (A), resulting in improved Water Use Efficiency (WUE). Under drought, BS-treated vines maintained higher E and A, further enhancing WUE and photosynthetic recovery. These trends were confirmed by high-temporal-resolution pot gravimetric data throughout the experiment.

BS induced a strong decrease in stem water potential and a marked increase in leaf osmolyte and polyphenol levels, reaching values typical of severe drought, even under irrigation. Stem starch reserves significantly increased in both WW and WS conditions. Transcriptomic analysis revealed substantial reprogramming of primary and secondary metabolic pathways.

Overall, BS triggered morphological and metabolic changes consistent with a priming effect, involving osmotic adjustment, early water-saving and sustained carbon assimilation and storage, supported by a clear shift toward anisohydric behavior.

03 EVOLUTIONARY AND FUNCTIONAL BIOCHEMISTRY IN PLANTS

Expanding the scope of pyrenoid-based CO2-concentrating mechanisms to enhance plant photosynthesis

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Many photosynthetic species have evolved CO2-concentrating mechanisms (CCMs) to improve the efficiency of CO2 assimilation by Rubisco and reduce the negative impacts of photorespiration. As the majority of plants (i.e. C3 plants) lack an active CCM, introducing a functional heterologous CCM into crops is a key engineering ambition to enhance yield potential. Most algae, including the green alga Chlamydomonas reinhardtii, possess a pyrenoid-based CCM that and enhances CO2 concentrations in the chloroplast and aggregates Rubisco into a liquid-like phase separated condensate resulting in faster catalysis and decreased photorespiration. Through advances in our understanding of the Chlamydomonas CCM, and more recently those in others species, the complex task of building a functional pyrenoid-based CCM in crops has moved several steps closer to reality, particularly now with a model-based roadmap to guide future engineering efforts. I will outline how pyrenoid-based CCMs can boost plant performance and resilience to climate change and our recent progress in transferring key components and features into plant chloroplasts.

From desert to seabed: Nature-based Engineering of Photosynthetic light harvesting systems

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Photosynthetic organisms, which feed on light, fuel Biosphere to produce biomass and oxygen by reducing atmospheric CO2. Light harvesting proteins (LHCs) bind 70% of total chlorophyll and carotenoids on Earth to catalyse both photon harvesting and photoprotection against oxidative stress caused by excess radiation. Photoprotection mechanisms confer resistance vs everchanging environmental conditions which are responsible for 82% crops productivity losses. Also, leaves bind chlorophyll in excess respect to its optimal for photosynthesis, thus imposing a light gradient in canopies, which limits growth and productivity. Here, we report on the structure-function analysis of Photosystem I and II supercomplexes using genome editing, cryo-EM and ultra-fast spectroscopy for the identification of protein/chromophore domains in LHC proteins. The cases of desert plants Welwitschia mirabilis thriving in the desert of Namib and of the seagrass Posidonia oceanica, growing in the dim light at 40 m depth in seabed will be discussed in the context of engineering crops for enhanced photoprotection of the upper leaves and improved light used efficiency of lower, shaded, leaves. We show that small difference in chromophore-chromophore interactions are responsible for large differences in light use efficiency of plants. Changes in Chlorophyll coordination control partitioning of excitation energy. Analysis of photosystems from plants adapted to contrasting light environments allow identification of specific mutations which determine interchromophore interactions. These concepts are being used for synthetic biology of light harvesting systems to produce high yield crops which reduces land use by agriculture to favour biodiversity and wildlife.

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Protein Homeostasis and Chloroplast Quality Control: Mis-Localization as a Signaling Mechanism

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The dynamic remodeling of the proteome and the maintenance of protein homeostasis (proteostasis) are essential for proper development and functionality of cellular compartments. Disruptions in proteostasis can severely compromise cellular health, leading to the accumulation of misfolded or mis-localized proteins. In chloroplasts, proteostasis maintenance poses particular challenges due to their endosymbiotic origin, intricate sub-compartmentalization, and exposure to reactive oxygen species generated during photosynthesis. To counteract these challenges, chloroplasts rely on elaborate quality control systems, including molecular chaperones, proteases, and dismantling pathways. Plastid protein mis-localization or accumulation beyond stoichiometric balance exposes hydrophobic regions and intrinsically disordered transit peptides, posing a significant threat to cellular homeostasis. In these studies, we examined how chloroplast homeostasis machinery responds to i) overexpression of two plastid ribosomal proteins (PRPS1 and PRPL4), ii) accumulation of plastid precursor proteins in the cytosol, and iii) stromal mis-localization of the lumenal protein PsbO. Our findings highlight that imbalances in nuclear-encoded, plastid-targeted proteins disrupt cellular homeostasis and trigger compensatory responses aimed at restoring chloroplast function or initiating organelle degradation. PsbO appears to serve as a pioneer protein during early plastid development. When correctly translocated to the thylakoid lumen, it supports chloroplast differentiation. However, if mis-localized, PsbO may function as a signal of plastid dysfunction, activating chloroplast dismantling.

Evolutionary Insights into β-Cyclocitral Signaling in Physcomitrium patens

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Carotenoids play a key role in plant signaling as precursors to phytohormones like abscisic acid and strigolactones. Among their oxidative derivatives, β-cyclocitral (β-CC) acts as a strong signal of photooxidative stress. Formed from β-carotene oxidation in chloroplasts under excess light (EL), β-CC triggers retrograde signaling and nuclear detoxification responses via SCL14, a GRAS-domain protein, and ANAC transcription factors, protecting cells from oxidative damage.

Yet, β-CC signaling remains elusive: it is volatile, water-soluble, lacks a known receptor, and no biosynthetic mutants exist. To explore its origins, we studied *Physcomitrium patens*, a bryophyte model offering insights into ancestral stress responses before vascular plant evolution. Using PAM fluorimetry, we found that under EL, β-CC reduced photosynthetic efficiency and increased ROS in *P. patens*, unlike its protective role in angiosperms.

Moreover, *P. patens* lacks a complete SCL14-regulated detox system, suggesting a transitional stage in β-CC response evolution. However, the strong transcriptional response to β-CC, largely overlapping with EL-induced genes, points to a conserved but functionally distinct signaling role in bryophytes. We also identified a direct, specific inhibition of photosynthesis by β-CC, absent in flowering plants, indicating an ancient signaling function later reprogrammed in vascular plants for optimized oxidative stress resilience.

Deciphering the redox regulation of the Calvin-Benson cycle enzyme fructose-1,6-bisphosphatase from Chlamydomonas reinhardtii

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Chloroplast fructose-1,6-bisphosphatase (FBPase) is a key enzyme in the regeneration phase of the Calvin-Benson-Bassham (CBB) cycle where it catalyses the breakdown of F-1,6-BP to fructose-6-phosphate (F6P) and Pi. Differently from its cytosolic counterpart, photosynthetic FBPase contains a regulatory loop bearing cysteines residues responsible for light dependent regulation *via* the ferredoxin-thioredoxin system.

This work consists of an extensive study of the recombinant photosynthetic FBPase from *Chlamydomonas reinhardtii*, with a particular attention to its redox-mediated regulation. It emerged that the enzyme dynamically shifts between two distinct quaternary states, each exhibiting completely different catalytic capacities. The redox-dependent activation was evaluated together with the midpoint redox potential.

The active form of the enzyme was isolated and its biochemical features (*i.e.*, turnover number, Michaelis-Menten constant and $S_{0.5}$) were determined, revealing a strong dependence on the enzyme's redox state.

Collectively, our results show that photosynthetic FBPase is subjected to several levels of regulation mainly mediated by redox signals, which are ultimately able to influence its catalytic properties. This paves the way to future studies aimed at the understanding of the specific role of individual cysteines in this complex regulation.

Mechanistic and redox-regulatory insights into plant aldo-keto reductase-mediated GSNO catabolism

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As an intracellular reservoir of nitric oxide (NO), S-nitrosoglutathione (GSNO) regulates its transport and biological activity. GSNO homeostasis is mainly controlled by GSNO reductase (GSNOR), but aldo-keto reductases of subfamily 4C (AKR4Cs) can also assist in this process. In this study, we characterized the biochemical properties of *Arabidopsis thaliana* AKR4C isoforms C8-C11, focusing on their kinetic features and redox sensitivity. These analyses revealed that AKR4C8 most closely resembles to GSNOR in terms of substrate/cofactor affinity and redox responsiveness. Using site-directed mutagenesis and heterologous expression, the importance of the catalytic tetrad in AKR4C8 (Asp43, Tyr48, Lys77, His117) was assessed, showing that each residue is essential for NADPH-dependent GSNO degradation. The redox sensitivity of AKR4C8 was examined after treatment with oxidizing agents like oxidized glutathione, hydrogen peroxide and GSNO. The analysis of cysteines accessibility identified Cys287 as the most likely oxidation site. Replacement of Cys287 with alanine did not affect enzymatic activity or structural integrity, but rendered the enzyme insensitive to oxidation, indicating that Cys287 is essential for redox regulation. Overall, our findings highlight that key residues in AKR4C8 are crucial for both catalytic activity and redox control, reflecting their central role in the enzyme's function. Future studies, including phenotypic analyses of Arabidopsis knock-out plants, will further elucidate the physiological role of AKR4C8.

Exploring photosynthetic diversity: the role of phylogeny and environmental plasticity

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Understanding how evolutionary history and environmental drivers shape photosynthetic diversity is key to predicting plant responses to global changes. Here, we explored the relative roles of phylogeny and plasticity in modulating leaf photosynthesis across 83 vascular plant species, representing 59 families and 33 orders, all cultivated under common garden conditions at the Botanical Garden of Padua (Italy). Leaf-level photosynthetic parameters were collected using a standardized protocol (Photosynthesis RIDES 2.0), repeated across seasons and daytimes.

Using phylogenetically informed ordinations (pPCA) and variance partitioning, we evaluated the contribution of evolutionary lineages versus acclimation. The photosynthetic trait space was largely conserved across major taxonomic groups, but specific traits, such as Fv'/Fm', NPQt, and Phi2, exhibited significant phylogenetic signals (Blomberg's K, Pagel's λ). However, species- and time-specific variation accounted for most of the intraspecific variability, highlighting a dominant role of short-term regulation.

Our results demonstrate that while some traits retain evolutionary imprints, phenotypic plasticity is the primary driver of functional diversity in photosynthesis. These findings support integrating broad phylogenetic sampling with high-resolution environmental measurements to better understand plant ecophysiology in a changing world.

Functional response of diatoms to a changing ocean chemistry

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In the context of a changing ocean characterized by acidification and global warming, significant efforts have been made to predict the physiological responses of diatoms, a productive and diverse group of marine phototrophs whose evolution of silica frustule may have played a critical role in global dominance.

Recent studies indicate that ocean acidification may lead to lower silicon (Si) concentrations in oceans, potentially causing a dramatic decline in diatom populations. Given the complex response patterns of diatoms to multiple stressors, this study aimed to evaluate the Si cellular demand of diatoms in future ocean conditions.

Diatom species of varying size and shape were acclimated to projected year 2200 seawater condition (at 23°C, 1000 ppm pCO2, and pH 7.5) and compared to cells acclimated to present-day environmental condition under Si-replete availability. We analysed cell growth, morphology, elemental and organic composition in these diatoms. The main results showed that under year 2200 condition a higher Si content per cell was found in comparison with that in present-day condition.

We repeated the experiment introducing Si limitation by changing the silicic acid concentration: 20 and 2 µmol L-1 of SiOH4 were used in the medium to represent present-day and future Si concentrations. Again, the Si content per cell was higher in response to expected temperature, pCO2, and pH.

Overall, Si limitation enhanced Si use efficiency and maintained biogenic Si production almost constant; nevertheless, the high plasticity level of diatoms could not avoid hampered growth suggesting adaptation to a future scenario may require higher energy cost of cell metabolism.

04 PLANT-ENVIRONMENT INTERACTIONS

Epigenetic memories in plants

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Epigenetics encompasses the complement of genetic information carried in chromatin beyond the DNA sequence. Studies in Arabidopsis, tomato and other plants, have demonstrated the relevance of epigenetic mechanisms in the control of development, impact on agronomic traits and genome stability. However, the transmission of epigenetic information through generation is still a matter of debate. So far, there is no comprehensive view on an epigenetic inter/trans-generational memory, which may vary depending on the factors inducing the epigenetic variations, the type of variations as well as on reproduction mode.

To investigate the transgenerational memory of epigenetic marks, we have used a unique tomato EpiRIL population generated by crossing WT and SlMET1 RNAi plants of the same genetic background. The population of EpiRILs consists of 115 lines that were propagated by single seed descent over 8 generations. Observable epigenetic and phenotypic differences are evident within the population. Methylome analysis of F4 and F7 plants of the EpiRIL population indicates methylation profiles in part corresponding to the WT or SlMET1 RNAi lines and provide evidence of inheritance of DNA methylation imprints over several generations. This EpiRIL population will now provide a valuable epigenetic community resource to evaluate the contribution of epigenetic variations to traits of agronomic interest including chemo/phenotypes and responses to environmental stresses. In addition, we are currently developing works in grapevine to investigate the consequences of (a)biotic stresses on the epigenomic landscape of plants, and to analyze the epigenetic memory of such stresses over years, and through clonal propagation.

Deciphering Plant Interactions with Friends and Foes: the VOC Language

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Plants produce and emit many volatile organic compounds (VOCs). Volatile isoprenoids, making more than half of the plant VOCs: a) interact with biogeochemical atmospheric cycles leading to the formation of ozone, particles and other air pollutants; b) protect the photosynthetic apparatus and maintain leaf integrity under abiotic stresses, namely water stress, high temperatures and oxidative stress; c) change the hormone balance affecting plant growth and biomass above/belowground allocation; d) drive plant communication with other organisms, eliciting direct and indirect defenses against herbivores and pathogens; and e) allow plant-plant interactions arguably priming naïve receivers to readily respond to incoming stresses. The latter function would require VOC receptors in receiving plants. However, currently only few VOC receptors have been discovered. How the bulk of VOCs is sensed by plants remains unknown and must be unraveled, to be able to use VOC priming as a sustainable plant protection practice.

The impact of rainfall regimes on barley seed development underlies genomic variation for germination after flooding

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Plant genetic diversity is partially shaped by adaptation to environmental stresses, with landraces and wild relatives serving as major sources of this variation. High precipitation levels can lead to soil flooding, which strongly impacts plant productivity and food security. Barley serves as a model species for studying the molecular responses of plants to climate change due to its wide adaptability and broad distribution across diverse environments. Barley has been shown to be particularly sensitive to flooding. This work examines how genetic variation in a global collection of barley landraces and wild relatives correlates with rainfall regimes in their environments of origin. We found a significant association between the rainfall patterns during seed development and the ability of barley accessions to germinate after flooding. Subsequently, we performed an environmental genome-wide association study (eGWAS) using exome sequencing data, identifying candidate genes potentially influencing germination responses to rainfall patterns. Through gene expression analysis, fluorescence-lifetime imaging microscopy (FLIM), and other molecular approaches, we demonstrated that genes linked hormonal regulation and seed morphology are essential for barley germination following flooding events. Our findings expand the genetic toolbox available to breeders and support the hypothesis that seed development is a key determinant of tolerance to germination after submergence in barley

Anterograde regulation of photosynthetic activity by the TOR kinase in plants

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Chloroplasts, essential for photosynthesis and energy production in plant cells, are also major consumers of energy and nutrients. To prevent energy waste and photooxidative damage, their activity must be tightly coordinated with overall plant growth. Here, we identify a posttranslational anterograde signaling mechanism that links the eukaryotic Target of Rapamycin (TOR) kinase that promotes growth and the guanosine tetraphosphate (ppGpp) signaling pathway of prokaryotic origin, which regulates chloroplast activity and photosynthesis in particular. We show that RelA-SpoT Homolog 3 (RSH3), a nuclear-encoded enzyme responsible for ppGpp synthesis in chloroplasts, interacts directly with the TOR complex in the cytosol through a plant-specific N-terminal domain that is phosphorylated in a TOR-dependent manner. Inhibition of TOR activity leads to rapid upregulation of ppGpp synthesis and a corresponding decline in photosynthetic capacity in an RSH3-dependent manner. These findings reveal a TOR–RSH3 signaling axis that modulates chloroplast activity in accordance with growth demands, highlighting a novel paradigm for TOR-mediated regulation of organellar function in plants.

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

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Keywords: Unfolded Protein Response, Mitochondria, Abiotic stress, *Arabidopsis thaliana*.

Mitochondria play a central role in cellular metabolism, from the production of energy to stress sensing and response. However, to preserve proper organelle function, it is necessary to maintain mitochondrial protein homeostasis. This is usually assured by the action of the mitochondrial protein quality control (mtPQC), a complex network of chaperones and proteases. Nevertheless, when misfolded or unfolded proteins accumulate beyond the capacity of this system, cells activate the mitochondrial unfolded protein response (UPRmt), a compensatory mechanism aimed at restoring proteostasis.

In this study, we identified new candidate UPRmt marker genes in Arabidopsis thaliana seedlings. Using these markers, we explored the involvement of UPRmt in response to various abiotic stresses. The data generated from this study provide evidence that UPRmt is a key component of the plant's adaptive response to environmental challenges, including but not limited to elevated temperatures, osmotic imbalances, and high salinity. These findings reveal the importance of UPRmt in abiotic stress resilience, potentially opening up new avenues for enhancing crop resilience in the face of climate change.

Hydrogen sulfide modulates plant responses to hypoxia via modification of the oxygen-sensing Plant cysteine oxidase (PCO) enzymes

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Hydrogen sulfide (H_2S) is a gaseous molecule known to participate to signalling of multiple physiological processes in living organisms. In plants, it interacts with hormones and other signalling molecules to regulate phenomena like stomatal closure, germination, maturation and senescence. Moreover, H_2S has been linked to plant defence and the response to abiotic stresses, including waterlogging, where H2S accumulation is favoured due to gas entrapment.
Here, we demonstrated that H_2S acts as a modulator of low-oxygen responses in Arabidopsis thaliana. Plants adjust their metabolism according to O2 availability, measured through Plant cysteine oxidase enzymes as dedicated sensors. Using biochemical and genetic approaches, we found that H_2S inhibits PCO activity through Cys persulfidation. Transcriptional and reporter assays further showed that this inhibition contributes to the modulation of hypoxia-associated molecular responses in vivo. Furthermore, we observed that physiological levels of H_2S are required to establish effective anaerobic responses. In the H_2S-deficient des1 mutant, indeed, full activation of hypoxia-inducible genes was prevented and tolerance to submergence was reduced. Our work expands the biological significance of H2S-mediated protein modifications, provides the first mechanistic explanation of the previously observed H_2S involvement in plant hypoxia physiology and reveals an additional layer of complexity of plant oxygen sensing.

Molecular dissection of the role of Aucsia-1 and Aucsia-2 in tomato root development

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Genome editing is an approach to accelerate breeding for crop improvement, particularly for plant species with well-characterized genetic and genomic resources, such as tomato. Genome editing allows customized modifications of target genes to achieve desired agronomic traits in a fairly predictable manner. Starting from a genetic resource identified in our laboratory, the *Aucsia* genes implicated in auxin-related fruit set and root development, we generated stable mutants of *Aucsia-1* and *Aucsia-2* in the tomato cultivar MicroTom using the CRISPR-Cas9 tool. The aim of this study is to dissect the role of the two genes in root development, with the future perspective of designing a tomato ideotype that combines the maintenance of high fruit set capacity with positive effects in root development, permitting adaptation to a changing environment. We obtained homozygous *aucsia-1* and homozygous *aucsia-2* mutants that appeared indistinguishable from WT plants in terms of shoot architecture and leaf morphology. The knock-out mutation of the *Aucsia-1* and *Aucsia-2* genes caused different alterations in root development. The *aucsia-1* mutants displayed an increased lateral root length, while the *Aucsia-2* mutation caused a reduction in lateral root density. Using *in vitro* tests and a hydroponic system, we evaluated whether the parsimonious root phenotype of *aucsia-2* mutants could confer an increased capacity to cope with salinity stress, and whether the changes in lateral root growth in the *aucsia-1* mutants can be advantageous under osmotic stress.

Auto-inhibited Ca^{2+}-ATPases ACA8 and ACA10 mediate wound-induced long-distance Ca^{2+} waves and ROS-dependent guard cell responsiveness in Arabidopsis

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The plasma membrane-localized auto-inhibited Ca^{2+}-ATPases ACA8 and ACA10 play a crucial role in cytosolic Ca^{2+} homeostasis and Ca^{2+}-dependent signaling in *Arabidopsis*. In our study, we demonstrate that both pumps contribute to maintain low basal cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) under resting conditions, as indicated by elevated basal $[Ca^{2+}]_{cyt}$ in *aca8* and *aca10* null mutant seedlings expressing the FRET-based Ca^{2+} sensor YC3.6. Moreover, using the red-shifted R-GECO1 Ca^{2+} indicator, we show that upon leaf and root wounding, wt seedlings exhibit rapid, long-distance Ca^{2+} wave propagation, whereas *both aca* mutants display impaired signal transmission, highlighting their key role also in systemic Ca^{2+} wave propagation. In contrast, by using the ROS-sensitive dye CM-H_2DCFDA, we detect intense ROS accumulation after wounding in *aca8 and aca10* mutants, with an intensity comparable to wt, suggesting that ACA8 and ACA10 are dispensable for wound-induced systemic ROS accumulation. Surprisingly, after injury and exogenous H_2O_2 treatment, stomatal closure is impaired in *aca* mutants, despite normal ROS production in guard cells, indicating that ACA8 and ACA10 are required to translate ROS signals into functional Ca^{2+} responses in stomatal regulation. Overall, our results support a model in which ACA8 and ACA10 act as key regulators of Ca^{2+} homeostasis and systemic signal propagation after wounding, thereby ensuring proper systemic communication and stomatal responsiveness.

Investigating shade tolerance in a S. pennellii introgression line.

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Global warming poses a critical threat to Mediterranean farming systems, requiring innovative cultivation strategies. One viable method is identifying tomato varieties that could be cultivated in intercropping systems (IC), in which two species grow together in shared spaces. Since leaves and other photosynthetic tissues absorb red light (R) but not far-red light (FR), under IC the light reflected from the leaves of neighbouring plants has a much lower R/FR ratio than direct sunlight. This low R/FR signal in shade-avoider plants results in elongated growth, increased apical dominance, reduced leaf number and size, premature flowering and degradation of photosynthetic pigments. Here the response to white (W) + FR of different tomato genotypes at seedling stage was compared allowing the identification of one *S. pennellii* introgression line with a shade-tolerant phenotype. Another experiment conducted during long-term exposure to W + FR confirmed the shade-tolerant phenotype of the IL. It was also demonstrated that the W + FR treatment improved tomato fruit quality by increasing °Brix and accumulation of ascorbic acid and carotenoids. Field trials under high density conditions further confirmed the shade-hyposensitive phenotype of the IL that also showed improved productivity. Genomic and transcriptomic investigations are under way that will allow for a more comprehensive characterisation of the response exhibited by the identified introgression line.

The root code cracked: deciphering the complex interplay between root exudation, drought and microbiota in wheat

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Keywords: root exudates, drought, peptides, soil microbiota

Plants employ sophisticated adaptive mechanisms to survive challenging conditions, including the active release of root exudates into the soil. Drought profoundly alters root exudate profiles, negatively impacting crops and their associated microbial communities. However, the precise modulation of this exudation remains poorly understood, with genetic factors (species, cultivars) likely contributing to this complexity. This study aims to unveil novel insights into how wheat plants adapt to **drought** by focusing on the **under-explored role of root exudates.** To address this, we developed a novel sterile semi-hydroponic system to collect root exudates after inducing water deficit. Subsequent **omic analyses** then allowed us to dissect drought-induced changes in exudate composition from two wheat cultivars with differing tolerances to water deprivation. Furthermore, comparative metagenomics was employed to elucidate shifts in rhizosphere microbial communities influenced by drought-stressed root exudates. Our results demonstrate that drought stress profoundly altered exudate profiles, leading to the release of specific compounds, including carbohydrates, phenolics, amino acids, and proteins. **Notably, we reported the drought-induced release of peptides in root exudates,** offering fresh perspectives on plant drought stress responses. We also observed cultivar-specific differences in exudate composition, directly linking them to their differential drought tolerances. Finally, the metagenomic analysis confirmed the complex interplay between root exudation, drought, and the plant's surrounding microbiota, providing a more comprehensive understanding of this critical tripartite interaction.

CRISPR-mediated knockout of SlMYB60 highlights functional divergence from its Arabidopsis ortholog

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Ongoing climate change and water scarcity threaten crop productivity, making drought resilience a critical challenge. Innovative strategies are needed to develop tomato varieties with improved water use efficiency and stable yield under water-limiting conditions.

The TOLERANT project (PRIN 2022 PNRR, P20228HKHM) targets ABA-mediated stress responses, focusing on *SlMYB60* (Solyc10g081490.1.1), the tomato ortholog of *AtMYB60*, a guard cell-specific transcription factor in Arabidopsis. To investigate *SlMYB60*'s function, CRISPR-Cas9 was used to generate knockout alleles in two tomato cultivars (Ailsa Craig and Red Setter). Edited plants showed a slight but significant increase in stomatal aperture. Under dry-down conditions, leaf area, fresh weight, and relative water content were significantly affected by both genotype and treatment.

RNA-seq revealed 186 differentially expressed genes in mutants, linked to stress responses, metabolism, and cuticle biosynthesis. Mutants exhibited increased cuticle permeability, altered epidermal surface, reduced guard cell density, and thinner stomatal ledges, indicating compromised water retention.

Expression studies using a *SlMYB60* promoter GFP:GUS fusion revealed activity beyond guard cells, suggesting broader roles in epidermal regulation. Our findings indicate that, unlike *AtMYB60*, *SlMYB60* is not guard cell-specific and affects epidermal traits, limiting its exploitation for improving drought tolerance in tomato.

Chemical genetics approach reveals new molecules as activators of plant hypoxic response

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Flooding events, aggravated by climate change, can impact growth and yield of crop plants, causing annual economic losses of several billion dollars over the previous 25 years. To counteract adverse weather events, the scientific community has continually sought solutions to improve agricultural performance. In this context, chemical genetics represents an innovative scientific approach for identifying and characterizing bioactive compounds that modulate plant physiological pathways.
In this study, we devised a high-throughput chemical genetics screening to evaluate the bioactivity of 2230 molecules, focusing on their ability to trigger an hypoxia-like response in *Arabidopsis thaliana*. Out of 2230 molecules screened, 3 were identified as potent activator of this pathway. We highlighted the ability of these compounds to transcriptionally promote genes related to the hypoxia response in plants. Furthermore*, in vivo* analyses demonstrated the unique ability of these molecules to promote this activity when compared to structurally similar molecules. Finally, a simulated waterlogging condition revealed that one of these compounds can promote a priming activity, improving plant tolerance to such stress conditions. These findings open the door to the agronomic application of this molecule to mitigate the devastating effects that flooding stress can have on crops.

Ultraviolet B radiations improve salt-induced responses in the facultative halophyte Chenopodium quinoa

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In natural environments, plants are continuously exposed to multiple abiotic stresses, with high salinity and excess light, e.g. ultraviolet (UV-B) radiations, being a key example. While responses to the individual stresses are well understood, less is known about their interactions. To address this, *Chenopodium quinoa* seedlings were salt treated (0 and 200 mM NaCl) under either photosynthetic active radiation (PAR), or PAR supplemented with UV-B radiation (313 nm, 1 hour/day, 1.71 W/m^2). While salinity had minimal effects on plant growth, it affected leaf gas exchanges and decreased photochemical efficiency. The addition of UV-B mitigated the negative effects of salinity and enhanced photosynthetic efficiency and water relations. This was associated with altered K^+translocation and compartmentalization in shoots. Indeed, while leaf K^+ remained high, its accumulation in epidermal bladder cells decreased. These results thus suggest that the improved performance in UV-B and salt-treated plants was linked to the redistribution of K^+ and water from epidermal bladder cells to the leaf tissues. This hypothesis was supported by the upregulation of both AKT1 (K^+ channel) and PIP1 (aquaporin) in UV-B and salt-treated plants. These findings provide critical insights into the interplay between UV-B radiation and salinity stress, highlighting a physiological adaptation that enhances plant resilience in salt-affected environments, particularly under conditions of increased UV-B exposure.

05 PLANT BIOTIC INTERACTIONS

Enhancing Pesticide Specificity Through Insights into Plant Wound Responses

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Insect decline represents a major societal challenge with profound ecological and economic consequences. Among the multiple drivers of this trend, pesticides are a critical factor contributing to biodiversity loss and disruption of ecosystem services. Addressing this challenge requires innovative approaches that increase the selectivity of pest control while minimizing harm to beneficial insects. To this end, we are investigating plant defense systems as a source of inspiration for developing targeted pest management strategies.

Our research focuses on the role of proteases in the plant wound response, where they function as key regulators of defense signaling and resistance. Previous work from our group identified metacaspases, a class of cysteine proteases, as enzymes that cleave small signaling peptides, including known damage-associated molecular patterns, placing proteolysis as a central mechanism in the activation of plant defense. Building on this theme, we are broadening the scope of damage-activated proteolysis to identify additional proteases, characterize their substrates, and elucidate their functions in regeneration post wounding.

Aiming to translate these insights into novel strategies for crop protection, we envision harnessing proteolytic activity to selectively activate the toxicity of pesticides, thereby restricting their action to herbivore attack sites and reducing collateral effects on non-target organisms. This work opens a path toward more sustainable and ecologically responsible pest management.

Enzymatic Oxidation of Cell Wall Oligosaccharides: Implications for Plant Defense and Cell Wall Remodeling

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Plant cell walls function not only as structural barriers but also as dynamic platforms for defense signaling. During pathogen attack, microbial enzymes degrade wall polysaccharides, releasing oligosaccharides such as oligogalacturonides (OGs), cellodextrins (CDs), xyloglucan-, mannan-, arabinoxylan-derived oligosaccharides, and mixed-linkage β-1,3/β-1,4-glucans (MLGs). These fragments act as damage-associated molecular patterns (DAMPs), triggering pattern-triggered immunity (PTI).

In Arabidopsis, the homeostasis of oligosaccharidic DAMPs is regulated by a family of oligosaccharide oxidases (OSOXs), including four OG oxidases (OGOX1–4) and two CD oxidases (CELLOX1–2), which also act on MLGs. These enzymes oxidize the reducing ends of oligosaccharides, simultaneously generating H_2O_2, which can also serve as a substrate for peroxidases (PODs) involved in lignin biosynthesis and auxin catabolism. Despite their structural similarity, OGOX isoforms show distinct developmental expression patterns, suggesting specialized roles in cell wall remodeling.

Fungal OSOXs have also been characterized and shown to suppress plant DAMP signaling by inactivating CDs and xylotetraose, emphasizing their role in plant–microbe interactions. Certain OSOXs can further redirect electrons from carbohydrates to oxidized phenolics, highlighting a functional link between cell wall and phenolic metabolism.

Importantly, altered OSOX expression in planta affects multiple developmental phenotypes, pointing to functions that extend beyond defense into plant growth and development. Together, our findings indicate that OSOX activity is central to balancing cell wall remodeling, redox homeostasis, and responses to environmental cues.

Further investigation is needed to elucidate the connections between OSOX activity, hormone signaling, and stress adaptation, with potential applications in biotechnology and crop improvement.

Deciphering HPCA1's role in OG-mediated defense and pathogen resistance

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Plants possess an innate immune system that relies on the ability to recognize danger signals, such as Microbe-Associated Molecular Patterns (MAMPs) and Damage-Associated Molecular Patterns (DAMPs), the latter including oligogalacturonides (OGs) derived from cell wall fragmentation. The local detection of these signals triggers Ca^{2+} influx and extracellular reactive oxygen species (eROS) accumulation. In the absence of a nervous system, plants rely on long-distance systemic signals to preemptively adapt to recurring stresses. ROS and Ca^{2+} waves play a key role in systemic communication between tissues, particularly in responses to wounding and abiotic stress. However, their involvement in systemic MAMP/DAMP signaling remains largely unexplored. The leucine-rich-repeat receptor-like kinase HPCA1 (H_2O_2-induced Ca^{2+} increases 1) is essential for eROS perception and the propagation of ROS/Ca^{2+} waves. While OG signaling has been extensively studied at the local level, the mechanisms governing its long-distance propagation and systemic response remain unclear. In particular, the role of HPCA1 in OG-induced immunity and pathogen defense has yet to be explored. In this study, we demonstrate that HPCA1 is required for both local and systemic responses to OGs and actively contributes to plant defense against various pathogens. Our findings establish a direct link between HPCA1 and OG-induced plant immunity, providing new insights into long-distance immune signaling in plants.

Xylem anatomical changes as adaptative response to long term Xylella fastidiosa infection in the susceptible olive cultivar Cellina di Nardò

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In 2013, *Xylella fastidiosa* subsp. *pauca* (*Xf*) was first detected in the Salento region of Apulia (South Italy) associated to the drying out of olive cultivars. Cellina di Nardò was among the most susceptible cultivars showing a rapid disease progression. In recent years, after more than 10 years of coexistence with the pathogen, the survived Cellina di Nardò plants seem to exhibit spontaneous canopy restoration. In this study, we investigated the adaptative strategies carried out by the olive trees to counteract the *Xf*. Our attention was focused on xylem anatomical traits usually affected in response to drought stress. A significant reduction in xylem vessel diameter and frequency was found, two traits associated with *Xf* resistance in several species. The new evidence concerns the vessel arrangement: a significant increase of solitary vessels. The replacement of the clustered vessels with the solitary ones reduces the contact points and, so, the inter-vessel connectivity of the xylem network, slowing down the *Xf* spread in the host plants and providing the ability to contain the pathogen. The laser scanning microscope observations of the *Xf* (labeled with the specific probe KO210) pattern distribution showed clearly the absence of bacterial vessel occlusions in plants with restored canopy. These results contribute to the knowledge of both the olive tree plasticity towards abiotic and biotic stresses and the *Xf* resistance mechanisms.

Submergence primes Arabidopsis thaliana for enhanced resistance to Botrytis cinerea

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Understanding how plants respond to sequential stresses has gained increasing attention in recent years. Flooding is an abiotic stress often followed by biotic challenges, as high humidity during the post-submergence promotes pathogen growth. Our research investigates how *Arabidopsis thaliana* responds to *Botrytis cinerea* infection following submergence. We found that submerged plants display enhanced tolerance to fungal infection compared to controls. In contrast, plants exposed to gaseous hypoxia rather than submergence are as susceptible to Botrytis as controls. Notably, exogenous ethylene application prior to infection restores the tolerant phenotype in both control and hypoxic plants. These results suggest that ethylene accumulation during submergence, but not under hypoxia, activates defense pathways that enhance pathogen resistance. Additionally, we identified a candidate gene involved in this response. This gene plays a role in pathogen resistance and is strongly induced following submergence and ethylene treatment. In untreated conditions, methylation of its promoter maintains a low basal expression of the gene. We propose that submergence triggers demethylation of the promoter region ensuring a proper induction of the gene. Taken together, our results reveal that flooding, through ethylene accumulation, activates the expression of specific regulators, leading to enhanced tolerance to pathogen infection after submergence.

14-3-3ε acts as a negative regulator of the response of Arabidopsis thaliana to Pst DC3000

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14-3-3 proteins are a family of conserved proteins which are widely present as multiple isoforms in all eukaryotes. They play regulatory roles by interacting with phosphorylated protein clients thus affecting their stability, activity or subcellular localization. In plants, these proteins are involved in the regulation of carbon and nitrogen metabolism, gene expression, ion membrane transport, hormone signalling and in the response to abiotic stresses such as drought, cold, and salt stress. We investigated a possible role of 14-3-3 proteins in the response to biotic stresses by infecting *Arabidopsis thaliana* plants with the model plant pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000. Interestingly, the levels of the transcripts of the 14-3-3 isoforms analysed decreased upon infection, with the ε isoform displaying the strongest reduction. Therefore, we studied the effect of the 14-3-3 ε overexpression in the Arabidopsis response to *Pst* DC3000. In particular, we evaluated the bacterial growth in the plant tissue, the stomata closure, the callose deposition, the expression of disease-related genes, and the development of symptoms on leaves. Results show that the overexpression of 14-3-3ε provides Arabidopsis with a heightened sensitivity towards the pathogen; so, we suggest that this protein might act as a negative regulator of plant immunity, keeping it on hold and being promptly downregulated upon pathogen perception.

Endophytes Allies Against Climate Change: Modulation of Growth and ABA Signaling in Arabidopsis thaliana Under Drought Stress

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Climate change is increasingly recognized as a major contributor to yield losses in agricultural crops, primarily due to its impact on environmental conditions. Enhancing plant tolerance to abiotic stresses is therefore essential to ensure plant survival and maintain yield quality. In this context, the beneficial roles of plant-associated bacteria and fungi represent a promising avenue for improving stress resilience. Here, we isolated endophytic strains from Sicilian grapevines adapted to arid conditions and investigated their potential to enhance drought tolerance in *Arabidopsis thaliana*.

In Arabidopsis plants, the inoculation with different endophytes led to observe effects on growth and stomatal aperture. Moreover, using transgenic Arabidopsis plants expressing the genetically encoded sensor ABACUS2-400, which allows *in vivo* monitoring of abscisic acid (ABA) levels, we observed that endophyte inoculation altered ABA levels in guard cells, suggesting a role in modulating stress hormone signaling.

These findings suggests that grapevines endophytes can influence the levels of this stress hormone in a different species, highlighting the potential to further explore their effectiveness in amelioration of stress responses.

Further experiments are underway to evaluate the impact of these endophytes on Arabidopsis drought tolerance, which could provide insights for future agricultural applications.

06 NEW FRONTIERS IN GREEN BIOTECHNOLOGIES

Practical Insights and Ongoing Challenges in Using Transgenics to Improve Plant Productivity

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Currently, both the agriculture and industry sectors are increasingly reliant on transgenic techniques and innovations. These technologies are also critical for investigating plant gene function and developing new crop varieties for the production of food, fuel, and high-value compounds such as therapeutic proteins and vaccines. Furthermore, it is estimated that between 2008 and 2050, yields of major food crops will need to increase by 70–100% to meet the demands of a growing global population. To achieve this, we will require a new "green revolution" in agriculture—one that will depend, at least in part, on transgenic technology.

In this talk, I will highlight photosynthesis as a promising target for boosting crop yields. I'll focus on work conducted as part of the RIPE consortium, showcasing how manipulating photosynthesis has led to measurable increases in plant productivity. I will also walk you through the transformation-to-phenotyping pipeline we used to generate some of our most successful transgenic lines.

Finally, I will touch on some often-overlooked but equally critical aspects of working with transgenics: not all modifications lead to the desired outcomes; challenges in achieving consistent and predictable transgene expression; and the fact that we still need to better understand how engineered plants will perform in complex environments—including their interactions with beneficial and harmful organisms, as well as their responses to abiotic stresses.

This talk aims to share not only progress and promise, but also some of the ongoing hurdles in deploying transgenics as a tool for improving crop productivity

Investigation of the Win-Win cooperation between C and N assimilation for biotechnological applications

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Agricultural nitrogen (N) fertilizers are currently provided by the Haber-Bosch process, highly energy demanding and responsible of approx. 2% of global energy consumption. In our fossil fuel economy, this drives to a large environmental impact and globally affects the eco-sustainability of food production. Diazotrophic cyanobacteria evolved the ability to fix atmospheric N2 into ammonium/ia, via a biological process powered by sunlight energy and have therefore the potential to be exploited as eco-friendly machineries for the sustainable production of N-fertilizers.

Past research efforts showed the feasibility of the approach, but they failed to achieve robust N excretion over time. This is because strategies that reduce the endogenous N assimilation flux curb also photosynthetic functionality and are thus expected to unbalance the energetic, redox and C/N status of the whole biological system.

The N assimilation pathway has been manipulated as if it were a standalone process so far, disregarding the intertwined connection with photosynthesis, that provides metabolic resources to the former (i.e. chemical energy, reducing power and C-skeletons). On the other hand, photosynthesis depends on pigments/proteins synthetized because of N assimilation, calling for a reciprocal functional coordination, that has never been investigated so far.

In this lecture I'm going to discuss our recent advancements in the dissection of the mutualistic functional relationship between C and N assimilation pathways, using the diazotrophic cyanobacterium Anabaena sp. PCC 7120, which evolved a spatial separation between the two metabolisms into distinct cell types.

Our research activity focuses on a twofold effort that combines spectroscopy techniques with next-generation metabolomics. In the first case, we exposed the biological system to variable metabolic inputs (e.g. light, CO2 and N availability) and investigated both photosynthetic functionality and nitrogen fixation to infer the contribution of the mechanisms for photosynthesis regulation in the functional coordination between C and N assimilation pathways upon changing environmental conditions.

On the other hand, we expressed genetically-encoded fluorescent probes to measure light-induced changes both of i) physical-chemical parameters (i.e. pH and redox status) and ii) the concentrations of universal carries of chemical energy (i.e. ATP), reducing power (i.e. NADH) and carbon skeletons (i.e 2-OG) to achieve high spatial and time resolution and determine their contribution in the functional coordination between the two assimilation pathways.

Exploiting the reducing power of cell wall oligosaccharides for the enzymatic synthesis of novel compounds

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Plant cell wall is a heterogenous matrix composed of polysaccharides, polyphenols, and proteins. The enzymatic hydrolysis of wall polysaccharides leads to the release of oligomers that can be oxidized by extracellular FAD-dependent oligosaccharide-oxidases (OSOXs) with the concomitant production of hydrogen peroxide (H_2O_2) from molecular oxygen. Here we show a novel reaction mechanism through which the oxidizing activity of OSOXs on short oligosaccharides, instead of producing H_2O_2, led to the reduction of bi-phenoquinones, suggesting a potential strategy to produce new compounds in an ecofriendly manner. By combining two additional enzymatic activities with the oligosaccharide-dependent reducing activity of OSOXs, a variety of novel molecules can be synthetized starting from plant waste material. This enzymatic system may represent a green alternative to conventional industrial methods for generating compounds with new chemical structures. The enzymatic mechanisms and reactions are discussed, highlighting their potential application in green chemistry and biocatalysis.

Antioxidant potential of halophilic microalgae from salt ponds of Trapani

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Keywords: Halophiles, Saltern ponds, antioxidant assay, Carotenoids, Metabolomics

Introduction

Halophilic microorganisms inhabiting hypersaline environments such as salt lakes, the Dead Sea, or salt evaporation ponds have evolved specific cellular adaptations to survive in extreme conditions[1,2]. This study focused on the microbial eukaryotic diversity of saltern ponds located within the Natural Reserve "Saline di Trapani e Paceco" in Sicily, Italy, with the aim of exploring their biotechnological potential.

Material and methods

Both culture-dependent and culture-independent methods were employed, including 18S rRNA metabarcoding, to assess the diversity and functional traits of eukaryotic microorganisms. Microalgal strains were isolated and identified, and their growth capacities were evaluated under varying salinity levels. Metabolomic profiling and pigment analysis of isolated microalgae were performed to assess their chemical diversity. Additionally, methanol extracts from cultured microalgae were tested for antioxidant activity using DPPH assay and cell repair activity in human cell line.

Results

New isolates were found to produce bioactive compounds with potential therapeutic applications. Notably, high salinity conditions were associated with enhanced production of antioxidant pigments such as β-carotene, and other carotenoids, and increased accumulation of metabolites like ectoine, betaine, amino acids, and lipids.

Conclusions

This work represents the first comprehensive study combining 18S rDNA metabarcoding and culture-based approaches to explore the eukaryotic microbial diversity in the 'Trapani-Paceco' saltern, highlighting its promise as a reservoir of bioactive molecules for biotechnological use.

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Anion Exchange Chromatography-Based Purification of Exosomes from Brassica oleracea L.: Molecular Profiling and Bioactivity in Human Cells.

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Plant-derived exosomes (PDEs) emerge as a promising alternative to mammalian-derived exosomes due to lower immunogenicity, enhanced bioavailability, and the presence of bioactive plant metabolites. They have been shown to cross biological barriers, delivering therapeutic molecules that modulate gene expression, inflammation, oxidative stress, and cancer-related pathways. However, challenges remain that limit applicative use, including poor knowledge of their interactions with mammalian host cells and the lack of a cost-effective and scalable purification methods for PDEs. To address these limitations, we developed an advanced purification platform combining ultrafiltration with anion exchange chromatography in a fast protein liquid chromatography system. This method was validated using it in the isolation of PDEs from *Brassica oleracea* seedlings, resulting in highly purified and concentrated preparations. Comprehensive molecular analyses, including proteomics, lipidomics, metabolomics, and miRNA profiling, confirmed their identity. Furthermore, their wound healing and anti-inflammatory properties were demonstrated *in vitro* and correlated to the potential biological activities of cargo miRNAs species by bioinformatics, therefore highlighting the potential in nanomedicine of anion exchange-purified PDEs. This study provides a scalable and efficient purification strategy, which might pave the way for broader applications of PDEs in clinical and nutraceutical fields.

Genetic engineering of Nannochloropsis oceanica to produce canthaxanthin and ketocarotenoids

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Canthaxanthin is a ketocarotenoid with high antioxidant activity, primarily produced by microalgae, among which *Nannochloropsis oceanica*. *N. oceanica* is widely used in aquaculture, can accumulate lipids up to 70% of dry weight and produce eicosapentaenoic acid (EPA), an ω-3 fatty acid used in food and feed. In this study, *N. oceanica* was engineered to overexpress the key enzyme for ketocarotenoid production, a β-carotene ketolase (CrBKT) from *Chlamydomonas reinhardtii,* to enhance ketocarotenoid and canthaxanthin production. The overexpression of CrBKT increased the content of carotenoids and ketocarotenoids per cell, 1.5 and 10-fold, respectively. At lower irradiances, the growth of *bkt* lines was slower compared to the background strain, while higher productivity was measured for *bkt* lines at 1200 μmol m^{-2} s^{-1}. However, the highest canthaxanthin and ketocarotenoids productivity were obtained upon cultivation at 150 μmol m^{-2} s^{-1}. *bkt* lines were also cultivated in 25L tubular photobioreactors under repeated batch regime, confirming the phenotypic stability and validating its potential for further scale-up toward industrial application. Through targeted gene redesign and heterologous transformation, ketocarotenoids and canthaxanthin content were increased, up to 0.3% and 0.2% dry weight, using CO_2 as the only carbon source. These engineered lines are promising candidates for fish and poultry feed sectors, where canthaxanthin and ketocarotenoids are required as pigmentation agents.

130 - Moon-Rice: cereal crop production for long term space exploration objectives

Vittoria Brambilla [(1)] *- Stefania De pascale* [(2)] *- Raffaele dello Ioio* [(3)] *- Marta Del Bianco* [(4)]

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The development of growth systems for cultivation in space aims at minimizing the resources required in terms of energy and volume. This technological progress affects also the plant organisms used, where the optimization of the plant architecture and productivity in a controlled environment will be fundamental to maximize space efficiency and reduce waste. In this context, for example, the generation and selection of crop dwarf varieties, which makes crops compatible with efficient vertical farming systems, is a rapidly expanding field for the first space settlements. An ideal space crop, however, should also be highly productive, optimised for growth in a closed environment and resistant to space stressors.

Most calories in the human diet come from complex carbohydrates, obtained mainly from the cultivation of cereals. Among cereals, rice is currently the main source of energy for the world population. Rice has interesting nutraceutical characteristics: it contains small and readily digestible starch, does not contain gluten and, depending on the variety, can provide fibre, proteins, vitamin B, iron and manganese. Rice is also one of the highest yielding cereals and can be easily grown with soilless methodologies (e.g., hydroponics), which are currently considered the most adapted technologies and potentially applicable to space exploration scenarios.

Starting from the identification of the ideal ideotype, the project Moon-Rice aims at developing new varieties of rice, selected specifically for future space applications.

POSTER PRESENTATIONS

01 PLANT HORMONES: FROM SIGNALING TO DEVELOPMENT

82 - Biological Control of Juvenility in Citrus: Insights into Tissue-Origin Effects in Somatic Embryogenesis

Angela Carra (1) - *Caterina Catalano* (1) - *Loredana Abbate* (1) - *Sergio Fatta Del Bosco* (1) - *Antonio Motisi* (1) - *Francesco Carimi* (1) - *Roberto De Michele* (1) - *Anna Maria D'Onghia* (2)

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Juvenility represents a major biological constraint in citrus regeneration via somatic embryogenesis (SE), often resulting in prolonged vegetative phases and delayed reproductive development.
This study investigates how different starting explant types (nucellar and stigma/style) affect the transition from the juvenile to the mature phase in SE-derived plants of four Citrus species.
Morphological markers of juvenility, including thorn length, thorn/node ratio, and absence of flowering, were monitored over three years in regenerants grafted onto sour orange rootstock.
Stigma/style-derived plants exhibited a significantly faster transition to the mature phase, with earlier flowering and fruiting observed within three years post-grafting. Conversely, nucellar-derived plants maintained strong juvenile traits.
Genetic stability of all regenerants was confirmed through flow cytometry and ISSR/RAPD analyses.
Results support the hypothesis that totipotent cells retain epigenetic memory from their origin tissue, influencing phase transition. Understanding these mechanisms can help reduce juvenility in SE protocols while preserving genetic stability.

144 - Transcriptomic Analysis Reveals an Estradiol-Responsive Pathway in Arabidopsis Involving EDS1 and RBOHD and Its Modulation upon B. cinerea Infection

Sara Di Renzo *(1) - Laura Guerrisi (1) - Valerio Licursi (2) - Giulia De Lorenzo (1)*

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Inducible promoters are invaluable tools in plant biology, enabling controlled modulation of gene expression while avoiding the potential negative effects of constitutive transgene activity. The XVE inducible system activated by β-estradiol is among the most widely used in plants. Although β-estradiol is generally regarded as biologically inert in plants, evidence suggests it may induce physiological changes, potentially through crosstalk with plant hormone signaling pathways. To investigate this, we performed a transcriptomic analysis of adult *Arabidopsis thaliana* plants at five days after β-estradiol treatment under both normal growth conditions and following infection with *Botrytis cinerea*. Our results show that β-estradiol induces substantial transcriptional reprogramming, affecting stress responses, immune pathways, and hormone signaling networks—including salicylic acid, jasmonic acid, ethylene, and abscisic acid. Moreover, β-estradiol treatment selectively modulates the plant's transcriptomic response to *B. cinerea* infection. Parallel analyses conducted in mutants lacking either the NADPH oxidase RBOHD or the immune regulator EDS1 revealed that both proteins contribute to the β-estradiol-influenced immune responses, both under basal conditions and after infection. These findings highlight the often overlooked biological effects of β-estradiol in plants and underscore the importance of accounting for its influence when employing it as an inducer in experimental studies.

02 CUTTING EDGE APPROACHES IN PLANT BIOLOGY

6 - Synthetic biology to investigate in vivo N-terminal cysteine oxidation of plant proteins

Noemi La Monaca *(1) - Mikel Lavilla-Puerta (2) - Pierdomenico Perata (1) - Beatrice Giuntoli (3)*

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Plants perceived molecular oxygen (O_2) through the Cysteine/N-degron pathway (Cys-NDP), where plant cysteine oxidase (PCO) enzymes using O_2 as co-substrate, trigger the oxidation of the N-terminal Cys from target proteins, leading them to proteasome-mediated degradation. Over 200 Met-Cys- (MC-) starting sequences can be derived from the *Arabidopsis thaliana* reference proteome, even if only seven of them have been found as PCO substrates therefore far, implying the potential existence of undiscovered substrate proteins. To facilitate the quick identification of novel MC- substrates *in vivo*, we developed a synthetic platform that enables the Cys-NDP for selective protein degradation in *Saccharomyces cerevisiae*, by co-expressing PCO enzymes in yeast and MC- protein sequences in proper reporter construct. With this approach, we analyzed a subset of sixty-four reviewed entries from the Arabidopsis MC- proteome and attained the identification of twenty-two novel targets. The positive candidates were afterwards transformed into Arabidopsis protoplasts, confirming the Cys-NDP dependence for fourteen of them, suggesting that heterologous screening provides reliable information on the regulation *in planta*. The novel MC- candidates identified, which include enzymes, signalling proteins and transcription factors, represents a valuable resource to enhance our understanding of how low O_2 levels regulate plant responses during stress (e.g., flooding), growth and development.

29 - StrigoSense: designing a novel biosensor for mapping strigolactone responses in plants.

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Strigolactones (SLs) are plant hormones involved in plant environmental interactions and development. Their spatiotemporal distribution is difficult to map due to their very low concentrations, hence the development of genetically encoded biosensors. Yet, all SL biosensors suffer from technical limitations, namely being negative sensors whose signal is degraded in response to SLs. With StrigoSense, we aim to construct a novel biosensor, which is positively activated by SLs, and whose output signal can be user-defined.

Our sensor module is composed of the D2 domain of the SL-responsive protein SMXL6 fused to the antiCRISPR AcrIIA4 protein, and of a dCas9-based transcriptional activation system (dCasEV). A user-defined reporter module comprises the reporter gene(s) of choice, controlled by a synthetic promoter containing the target sequence of the CRISPR-based activation system. Thus, in the absence of SLs, the antiCRISPR protein inhibits dCasEV activity, maintaining the reporter signal off. When SLs increase, the SL-dependent polyubiquitination of D2 also leads to the degradation of the antiCRISPR protein, and the dCasEV system can boost reporter transcription.

We will develop the StrigoSense biosensor with fluorescent and bioluminescent reporter systems for revealing single-cell or system-wide responses. StrigoSense will be developed and tested in Arabidopsis and tomato, seeking to move from model organisms to crops and towards biotechnology-based agricultural solutions.

60 - Nonstructural role of fibers in poplar during drought

Niccolò Tricerri (1) *- Martina Tomasella (2) - Silvia Cavalletto (1) - Francesco Petruzzellis (3) - Alan Crivellaro (1) - Rachele Gamba (1) - Alma Piermattei (1) - Lorenzo D'Amico (4) - Giuliana Tromba (4) - Andrea Nardini (2) - Maciej Zwienieki (5) - Francesca Secchi (1)*

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Vessel recovery from embolization events is a mechanism important for long-term tree survival and fitness. The process requires osmolytes and water, the former supplied mainly by parenchyma cells while water can be obtained from multiple sources. Fibers in addition to their structural role are also a reservoir of unbound water but how easily it can be accessed remains unclear. By imposing two drought regimens (fast, 7 days or slow, 21 days) on *Populus nigra* plants we observed different usage of fibers water. Synchrotron micro-computed tomography reconstructions showed no difference in vessel embolism formation but slowly stressed plants accessed more of the fiber-stored water. Interestingly, after stress is relieved, fast-stressed plants were the group that maintained better fiber hydration levels and also better recovered embolized vessels. SEM anatomical observations showed no direct connection between fibers and vessels while simple pits are present towards parenchyma cells creating a preferential path. Through physiological evidences and anatomical analyses we propose a novel functional role for fibers in Poplar during drought events. Our data suggest that parenchyma cells can access fibers water to ensure living tissue survival under prolonged water deprivation, the same water can be utilized to favor hydraulic recovery when drought ends if it was not required before.

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64 - From Petri Dish to Natural Habitat: Development of New Technologies to Study How Plants Respond to Environmental Cues

Stefano Buratti *(1) - Bianca Maria Orlando Marchesano (2) - Nour Djihane Bouferkas (2) - Francesca Resentini (2) - Giorgia Tortora (3) - Andrea Bassi (3) - Maria Cristina Bonza (2) - Alex Costa (2)*

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Being rooted in the soil, plants have evolved sophisticated mechanisms to perceive, transduce, and respond to environmental challenges. In response to both biotic and abiotic stresses, they employ specific receptors, hormones, signaling molecules, second messengers, and ions movements across membranes (e.g., Ca^{2+}) mount tailored responses. In recent years, the use of genetically encoded fluorescent indicators combined with fluorescence microscopy has revolutionized the field, enabling *in vivo*, real-time visualization of these players.

However, imaging experiments typically rely on young seedlings or isolated plant tissues (e.g., leaf discs) due to size constraints imposed by standard microscopy setups. These simplified systems may fail to fully capture the complexity of whole-plant responses under realistic conditions.

To overcome these limitations, we have developed a custom-made microscopy setup that allows *in vivo* imaging of genetically encoded fluorescent indicators in soil-grown Arabidopsis plants. To complement this platform, we created 3D-printed, customizable devices integrating open-source electronic sensors. These tools enable precise control and monitoring of soil parameters while applying environmental stimuli directly to plants grown in near-physiological conditions.

Using these systems, we successfully visualized Ca^{2+} dynamics in GCaMP3-expressing Arabidopsis plants during flooding, hypoxia, and osmotic stress. Our setup enabled reliable side-by-side comparisons between wild-type and mutant lines, reducing artifacts associated with traditional, reductionist approaches.

Altogether, this work presents a powerful, flexible toolbox to study how plants perceive and respond to environmental cues under conditions that better reflect their natural habitat.

67 - APPI: a Versatile Platform for Whole Plant Imaging

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Unable to escape from unfavourable conditions, plants evolved complex mechanisms to perceive and respond to various environmental stimuli using signaling molecules and second messengers. *In vivo* imaging experiments on Arabidopsis plants expressing Genetically Encoded Fluorescent Indicators (GEFI) revealed that mechanical stimuli trigger long-distance apoplastic glutamate and cytosolic Ca^{2+} waves propagating from leaf-to-leaf or root-to-leaf.

These signals are essential for activating stress marker genes and hormone synthesis in systemic tissues. While Arabidopsis has been key in uncovering these processes, it remains unclear whether such mechanisms are conserved in larger plants. Thus, we investigated adult *Nicotiana benthamiana* plants expressing different GEFIs to *in vivo* study Ca^{2+} and glutamate dynamics. To image *N. benthamiana* plants, we developed the Adult Plant Projective Imaging (APPI) platform, a custom-made large field-of-view fluorescence imaging setup, that allows the simultaneous orthogonal imaging of shoot and root of plants with sizes up to tens of centimetres. Thanks to the APPI system we were able to confirm the existence of local and systemic Ca^{2+} and glutamate signals propagation in the shoot in response to mechanical damages and insect chewing. By leveraging the orthogonal view and customized 3D-printed supports, we visualized shoot-to-root Ca^{2+} signals triggered by mechanical damage, revealing communication between above- and below-ground plant's organs.

116 - Artemisia at flowering: optimizing in planta accumulation and in vitro evaluation of secondary metabolites with potential anticancer activity

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Since ancient times, humans have used medicinal plants for therapeutical purposes. Still today, a quarter of pharmaceuticals employed in modern medicine have a plant origin. Nevertheless, biomolecules of interest often accumulate at very low levels in specific structures or at specific developmental stages, thus hindering their large-scale production in plants. For example, *Artemisia annua* – an aromatic plant used as herbal remedy in Traditional Chinese Medicine and recommended by WHO as antimalarial medication – produces the key compound Artemisinin (AN) only in leaf trichomes at flowering, making its biosynthesis difficult in the quantities needed for therapeutic results. Previous research revealed that treatment of *A. annua* with bio-stimulants not only accelerates the plant life cycle but also enhances AN content by increasing the number of trichomes. Besides AN, *A. annua* leaves accumulate other compounds with potential antioxidant, anti-inflammatory and antiproliferative properties.

Ongoing research aims at optimising the extraction of secondary metabolites from dried leaves of *A. annua* for *in vitro* testing. By using 3D models recognized as standard for the tumorigenic potential of malignant cells, we are evaluating the anticarcinogenic capacity of different plant extracts. We are also investigating the possible ethnobotanical convergence among related species of the *Artemisia* genus with different geographical origins currently growing at our botanical garden.

134 - LHCSR1 functions as a dimmer switch in membrane nanodiscs

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Under high light, photosynthetic organisms dissipate excess energy non-radiatively in a set of processes known as non-photochemical quenching. In moss and algae, high light conditions induce the thylakoid lumen acidification, triggering the activation of the light-harvesting complex stress-related proteins (LHCSRs) and converting the LHCSR-bound xanthophyll violaxanthin into zeaxanthin. Previous studies of LHCSR1 isolated in detergent found that pH and zeaxanthin binding regulate two distinct quenching pathways. However, it is not known whether this behavior is maintained in a lipid environment. In this work, we employ single-molecule fluorescence spectroscopy to find that the energetic landscape is flattened in a membrane environment, suggesting that conformational changes induced by the environment regulate the transition pathways between quenched and active states.

148 - High-throughput phenotyping to study biostimulant efficacy in triggering plant responses to drought stress

*<u>**Marcello Deandrea**</u>* ***(1)*** *- Davide Lucien Patono (1) - Filippo Garis (1) - Martina Tarditi (1) - Alessandra Ferrandino (1) - Claudio Lovisolo (1)*

(1) Università degli Studi di Torino, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Grugliasco, Italia

Advanced high-throughput phenotyping technologies were used to study how biostimulants (BS) influence plant responses to drought stress. Experiments at the PhenoPlant platform (UniTO) involved multiple crops and BS prototypes. In maize, drought progression led to canopy reduction due to leaf rolling. A slower decline in dimensional indices, detected through 3D imaging, revealed that some BS delayed stress symptom onset. In grapevine, 3D imaging identified leaf angle as a sensitive drought indicator, with BS mitigating its reduction. Automated high-resolution gravimetric data showed daily water loss over pot weight reliably captured BS effects, serving as a proxy for root water uptake ability. In lettuce, treatments reduced the shoot/root ratio without affecting final shoot biomass. This dynamic was revealed by dimensional and transpiration data, showing an initial decline followed by improvement, as treated plants closed the growth gap under severe stress and subsequent recovery by maintaining higher transpiration. This suggests early root allocation and improved soil exploration under stress. High-throughput results were validated by direct measurements of water and osmotic potential and starch content, supporting the link between BS responses and improved osmoregulation and changes in carbon allocation. Overall, the study highlights the value of high-throughput phenotyping in identifying drought-mitigation traits triggered by BS.

03 EVOLUTIONARY AND FUNCTIONAL BIOCHEMISTRY IN PLANTS

5 - Dissecting the multiple functions of LHCb proteins in photosynthetic light use efficiency and photoprotection.

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(1) Università di Verona, Biotecnologie, VERONA, Italia - (2) Aix Marseille University, CEA, CNRS, LEMiRE, Marsiglia, Francia - (3) HUN-REN, Biological Research Centre, Szeged, Ungheria

In higher plants, Photosystems (PS) I and II operate in series to ensure high photosynthetic performance. This is achieved through the dynamic regulation of exciton lifetime and the structural remodelling of thylakoid membranes in response to ever-changing environmental conditions. Excess illumination induces ROS formation in the chloroplasts, thus causing PSII photoinhibition. To cope with this, plants developed a series of rapid mechanisms aimed at dissipating excess excitation as heat, known as Non-Photochemical Quenching (NPQ). Key players in both thylakoid light-dependent remodelling and NPQ reactions are the Light-Harvesting Complexes (LHC). However, the specific contributions of individual LHCs to these physiological responses are still to be fully elucidated.

In this study, we dissected the PSII antenna system *in vivo* by generating *Arabidopsis* lines devoid of specific LHC subgroups: monomeric Lhcb4-5-6, trimeric Lhcb1-2-3, or both. Electron Microscopy and Time-Resolved Fluorescence analyses revealed that both monomeric and trimeric LHCs were essential for ensuring the optimal organization of thylakoid membranes and preventing energy spillover from PSII to PSI. Dynamic remodelling of thylakoid architecture mainly relied on trimeric component Lhcb2, whereas monomeric Lhcb5 was critical in stabilizing thylakoid stacking. Trimeric complexes showed the highest contribution in NPQ induction, while monomeric Lhcbs conferred the strongest photoprotection under excess light. Our findings pinpointed distinct roles of LHC subgroups in membrane architecture, energy dissipation, and photoprotection.

11 - Coordinated photosynthesis and respiration sustain cellular metabolism and architecture

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Mitochondria and chloroplasts act as cellular hubs, engaged in crosstalk through energy exchange, carbon intermediates, and regulatory signals that coordinate photosynthesis and respiration. However, the pathways underlying this functional and structural communication are not yet fully understood.

In this study, we exploited the moss *Physcomitrium patens* to isolate mitochondrial OXidative PHOsphorylation System (OXPHOS) mutants normally non-viable in other plants. Mutants lacking respiratory Complex I (CI), Complex IV (cytochrome c oxidase), or Complex V (ATP synthase), demonstrate that mitochondrial respiration is not merely a backup for night-time or heterotrophic metabolism but is central in photosynthetically active cells. Its disruption causes severe growth defects, altered photosynthetic electron transport, and metabolic shifts, including reduced carbon fixation and amino acid biosynthesis.

Structural data show that impaired respiration disrupts energy metabolism and leads to cellular disorganization, impacting both chloroplasts and mitochondria. OXPHOS mutants display altered organelle morphology and distribution, impaired starch metabolism, and plastoglobuli accumulation, hallmarks of cellular stress. These findings highlight mitochondrial respiration's broader role in maintaining organelle integrity and homeostasis, essential for optimal metabolism and bioenergetics. Interestingly, the observed impact of respiration inactivation is larger than in green algae, suggesting that key processes related to energy regulation, signaling, and stress responses may be more tightly integrated with mitochondrial function.

33 - Dynamic regulation of Arabidopsis β-amylase1 by glutathione and thioredoxins affects starch in guard cells

Libero Gurrieri (1) *- Anna Clara Capuzzi* (1) *- Stefanie J. Müller-Schüssele* (2) *- Paolo Trost* (1) *- Francesca Sparla* (1)

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Guard cells control the opening and closure of stomatal pores in response to internal and external stimuli, ensuring gas exchange in plants. In *Arabidopsis thaliana*, β-amylase 1 (BAM1) starts degrading starch at dawn in guard cells to provide carbon skeletons essential for stomatal opening.

Given that thioredoxins regulate BAM1 activity, we investigated its sensitivity to other redox-dependent modifications. Recombinant BAM1 is reversibly inactivated by H_2O_2, which, in the presence of glutathione, induces *S*-glutathionylation of BAM1. Glutathionylated BAM1 is active and transiently protected from H_2O_2 inhibition. However, the glutathionylated state of BAM1 is unstable and a second cysteine attacks the glutathionylated cysteine leading to the formation of an intramolecular disulfide bond. This disulfide bond inhibits BAM1 and thioredoxin *f* restores the activity.

Arabidopsis plants with lower plastidial glutathione reductase activity, and consequently modified glutathione homeostasis, showed higher BAM1 activity, lower starch levels in guard cells and altered stomatal aperture, indicating that glutathione redox potential impacts stomatal physiology, possibly through BAM1.

BAM1 presents a prime example of glutathionylation as a transiently protective modification interfering with the formation of an inhibitory disulfide bond. It illustrates how transitions between cysteinyl thiol modifications can orchestrate dynamic responses involving several redox systems.

52 - Modulating phosphoribulokinase affinity: A Proof-of-Concept Study

Anna Clara Capuzzi (1) *- Alix Matinet* (1) *- Matteo Calvaresi* (2) *- Edoardo Jun Mattioli* (2) *- Silvia Fanti* (2) *- Simona Fermani* (2) *- Paolo Trost* (1) *- Francesca Sparla* (1) *- Libero Gurrieri* (1)

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BAM1 presents a prime example of glutathionylation as a transiently protective modification interfering with the formation of an inhibitory disulfide bond. It illustrates how transitions between cysteinyl thiol modifications can orchestrate dynamic responses involving several redox systems.

70 - NADPH metabolism shapes plant stress responses and reproduction

Elisa Dell'Aglio **(1)** *- Fabio Palmigiani (1) - Sara Paola Nastasi (1) - Laura Luoni (1) - Stefano Buratti (2) - Francesca Resentini (1) - Alex Costa (1)*

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The NAD(P)/H redox balance is a fundamental regulatory mechanism that directs metabolic reactions, development and stress responses. However, detailed spatial and temporal regulations of the NAD(P)/H balance and the molecular players responsible for this regulation are still poorly characterized. Among different actors, enzymes that convert NAD^+ into $NADP^+$ play an important role.

In all organisms, the only enzyme able to produce $NADP^+$ is the NAD^+ kinase (NADK). We have recently discovered that in the green lineage (plants and green algae) some isoforms of NADK are calcium and calmodulin dependent. In *Arabidopsis thaliana*, this regulation allows the plant to promptly adapt its NAD(P)/H pools to counteract stress conditions and initiate appropriate signaling cascades, such as the Reactive Oxygen Species (ROS) burst produced in *Arabidopsis* seedlings in response to pathogen elicitors. Interestingly, in Arabidopsis other calmodulin-dependent NADKs are present, and our data show that they are specifically expressed in mature pollen, where they seem to be important to counteract the effects of high temperature. Thus, this pushed us to investigate when, during the plant lineage evolution, this type of calmodulin-dependent NAD^+ kinase appeared, and what could be their physiological role Noticeably, our analyses has revealed that genes coding for calmodulin-dependent NAD^+ kinases are already present in green algae and basal plants like *Marchantia polymorpha*. Our biochemical analyses confirmed their function as NAD^+ kinases, and we are now exploring the conservation of their physiological roles in these species. Our preliminary results suggest that the NAD(P)/H redox balance is a pivotal node of primary metabolism at the center of several plant adaptative responses.

72 - Post-translational modifications in plant and algae enzymes: new insights in the redox regulation of plastidial Glucose-6-Phosphate Dehydrogenase from Populus trichocarpa and Chlamydomonas reinhardtii

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Glucose 6-phosphate dehydrogenase (G6PDH) plays a key role in regulating carbon flux through the oxidative pentose phosphate pathway (OPPP).

The generation of NADPH moieties by the G6PDH (and 6P-gluconate dehydrogenase) reaction is crucial for plant metabolism, maintaining cellular redox homeostasis, and stress response.

In plants, plastidial G6PDH isoforms are regulated by a disulfide bond that can be physiologically reduced by thioredoxin *m*.

In this study, we investigated the possible occurrence of post-translational modifications in different cys residues present in *Chlamydomonas reinhardtii* and *Populus trichocarpa* plastidial G6PDH isoforms.

By using cys-mutagenized recombinant enzymes, we found that cys residue(s), adjacent those involved in regulatory disulfide, could be nitrosylated, thus exercising an inhibition of plastidial G6PDH activity. Intriguingly, $NADP^+$ exerts a protective effect on nitrosylation, indicating a high complexity in the modulation of G6PDH activity in plastids.

Hopefully, these results aim to contribute to a better understanding of the basal mechanisms regulating the primary metabolism in plants.

103 - Preliminary Studies on the Interaction Between Thioredoxin and Phosphoribulokinase

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Phosphoribulokinase (PRK) is a key enzyme in the Calvin-Benson cycle, regulated by thiol-disulfide exchange. Previous studies have shown that reduced thioredoxin f (Trx f) activates oxidized PRK through the formation of an interprotein disulfide intermediate, where Cys46 of Trx f transiently pairs with Cys55 of PRK. Although the Trx-dependent reduction mechanism is well characterized, the recognition process between the two proteins is unknown.

To shed light on this process, the 3D structure of the intermediate binary complex between PRK and Trx f must be solved. To this aim, *Spinacia oleracea* PRK and Trx f were cloned into the expression vectors pRSFDuet and pET28 and Cys17 and Cys 49, respectively, were mutated into Ser. Such mutations are required to obtain a stable PRK-Trx f binary complex.

The two mutated recombinant enzymes, PRK C17S and Trx f C49S, were purified and complex formation *in vitro* was tested under different conditions. To activate Cys residue, PRK C17S was treated with a 5-fold excess of DTNB for about 1h. Subsequently, DTNB-activated PRK was incubated with Trx f C49S at four different molar ratios, and complex formation was assessed by SDS-PAGE.

Preliminary results suggest that a ratio of 1 PRK C17S per 1.2 Trx f C49S is optimal for complex formation. However, some unbounded PRK C17S remained into the sample, making it not suitable for the subsequent 3D analysis. Therefore, purification protocol and interaction conditions must be optimized.

125 - Cytochromes b561 and ascorbate redox homeostasis: a hidden relationship

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Cytochromes b561 (CYB561s) constitute a wide family of Eukaryotic proteins with conserved architecture, transmembrane structure and capability to transfer electrons from cytosolic ascorbate (Asc) to extracytosolic electron acceptors, either the oxidized form of ascorbate, monodehydroascorbate, or ferrichelates. Arabidopsis contains four isoforms of CYB561 (from A to D) and more than 10 CYB561-containing proteins named CYBDOMs. The intracellular localization of CYB561-related proteins is complex and not fully elucidated, but tonoplast, ER, Golgi and plasma membrane are all membranes reported to contain at least one CYB561 or CYBDOM isoform. CYB561-A was recently shown to work as a trans-tonoplast, Asc-dependent MDHA-reductase in Arabidopsis vacuoles with suitable kinetic features for the reciprocal regulation of cytosolic and vacuolar Asc redox states. Knock-out mutants of CYB561-A, but also of CYB561-B or SDR2 (a CYBDOM isoform), all contain normal levels of Asc and normal Asc redox states in total leaf extracts, but tend to share a series of similar, Asc-related phenotypic traits. These include: (i) lower ROS content, (ii) higher ascorbate peroxidase activity, (iii) impairment of nonphotochemical quenching (iv) inhibition of primary root growth, (v) marked delay in flowering onset and (vi) hyperaccumulation of anthocyanins under strong light. A local alteration of the Asc redox state is suspected to be the common cause of the shared pleiotropic phenotypes of the mutants.

146 - In vitro antifungal and antibacterial activity of exosomes purified from Brassica oleracea L.

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Exosomes are the smallest subset of extracellular vesicles ranging from 200 to 20 nm. They have the ability to carry and deliver a variety of bioactive molecules, including proteins, lipids, and small RNA. In our study, plant-derived exosomes (PDEs) were purified from *Brassica oleracea* L. sprouts using an innovative purification protocol geared for industrial applications. The protocol combined ultrafiltration, enzymatic digestion, and anion-exchange chromatography with an FPLC system to achieve high purity and yield PDE preparations. Purified PDEs, stained with the lipophilic dye FM4-64, were visualized using a confocal Laser Scanning Microscope confirming the successful isolation and the vesicles size in the expected nanoparticles range. Microbiological assays were conducted to *in vitro* test the antimicrobial activity of the PDEs. The PDEs were tested against three pathogens that are causative agents of important diseases of herbaceous and woody species: the fungi *Botrytis cinerea* and *Alternaria solani,* and the bacterium *Xylella fastidiosa*. The obtained results showed the ability of the PDEs to significantly inhibit the *in vitro Botrytis cinerea*, *Alternaria solani and X. fastidiosa* growth. These results demonstrate the potential of PDEs to treat these infections *in vivo;* therefore, further tests will be conducted on infected plants to confirm the detected antimicrobial activity and the content of purified exosomes in bioactive molecules will be characterized.

04 PLANT-ENVIRONMENT INTERACTIONS

3 - Abiotic stress-induced chloroplast and cytosolic Ca2+ dynamics in the green alga Chlamydomonas reinhardtii

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Calcium (Ca^{2+})-dependent signalling plays a well-characterized role in the perception and response mechanisms to environmental stimuli in plant cells. In the context of a constantly changing environment, it is fundamental to understand how crop yield and microalgal biomass productivity are affected by external factors. However, the knowledge of Ca^{2+} signalling in green algae remains limited, and even if Ca^{2+} is known to be important in different physiological processes in microalgae, many of these signal transduction pathways still need to be characterized. Here, the role of compartment specific Ca^{2+} signalling was investigated in *Chlamydomonas reinhardtii* in response to a range of environmental stressors, such as high light, nutrient availability, osmotic stress, temperature fluctuations and carbon sensing. An *in vivo* single-cell imaging approach was adopted to directly visualize signalling processes at the level of specific subcellular compartments, using *Chlamydomonas reinhardtii* lines expressing a genetically encoded ratiometric Ca^{2+} indicator. Obtained data report cytosolic and chloroplast compartment-specific [Ca^{2+}] transients, characterized by stimulus-specific kinetic parameters. Moreover, a relevant role of the chloroplast Ca^{2+} signalling was identified in response to high light, hyperosmotic shocks, heat stress and different exogenous carbon sources. Together these data will provide new understanding of the mechanisms that microalgae exploit to respond to specific natural conditions.

4 - Uncovering key genes at the heart of salinity stress response in durum wheat

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Soil salinity is a major threat to crop productivity. Here, four durum wheat recombinant inbred lines (RILs$_{F7:F8}$) with contrasting responses to salt stress were selected based on germination rate, root and shoot length, and biochemical parameters such as ion content and photosynthetic efficiency. Genotype 158 displayed high tolerance, maintaining elevated germination rates and superior physiological performance under salt stress, genotype 15204R showed reduced germination and lower fitness while 1502R and 15209 exhibit intermediate levels of tolerance.

To investigate molecular mechanisms of tolerance, transcriptomic analysis was performed. Results revealed a similar number of DEGs between each genotype under control and stress conditions, but only a few of them were shared across genotypes, suggesting genotype-specific responses. In the tolerant genotype, upregulation of genes related to photosynthesis was observed, while in 15204R genes involved in ion homeostasis and ROS detoxification were downregulated. WGCNA allowed the identification of 35 co-expressed modules, among which three were strongly associated with salt stress, whereas other two with tolerance-related traits. Intramodular analysis revealed h**ub genes involved in secondary metabolism (e.g.,** PAL and PPO**) and** TFs belonging to different subfamilies, including ERF and WRKY.

Our findings highlight key molecular mechanisms underlying salt tolerance in durum wheat and suggest candidate genes for breeding strategies.

10 - Sun-kissed or sun-stressed? Investigating UV-B's impact on quinoa germination

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Chenopodium quinoa Willd. (quinoa) is a crop native to the Andean region of South America. This pseudocereal has garnered global attention due to its high nutritional content and adaptability to diverse environments. Changes in stratospheric ozone and climate are affecting ultraviolet (UV) radiation at the Earth surface, especially UV-B (280 - 315 nm), thus understanding how plants like quinoa respond to increased UV-B radiation is crucial for sustainable agriculture [1-3]. This study investigated the effects of UV-B exposure on the germination process of quinoa seeds, the most important growth stage that determines plant establishment. Employing controlled laboratory conditions, quinoa seeds were subjected to acute UV-B radiation, and biometric and physiological traits were monitored and analyzed during 24 h-treatment starting with seed imbibition. The results of this study will be discussed in this presentation, contributing to a deeper comprehension of quinoa's adaptive strategies in response to environmental stress with special focus in regions with heightened UV-B exposure due to climate change.

References:
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12 - Arbuscular mycorrhizal symbiosis impacts the regulation of salt response related pathways in tomato roots

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Arbuscular mycorrhizal fungi (AMF) have been reported to enhance salt tolerance in tomato, yet the underlying molecular mechanisms of the AMF-mediated salt tolerance in roots remain largely unexplored. Here, we investigated the root transcriptional responses of mycorrhizal and non-mycorrhizal tomato plants subjected to salt stress in controlled conditions. Results reveal that AMF colonization triggers a broader and more robust transcriptional reprogramming, mainly enhancing genes associated with Ca^{2+} signalling. AMF modulated salt-stress perception pathways, including the differential regulation of the tomato homolog of the *Arabidopsis* inositol phosphorylceramide glucuronosyltransferase *SlMOCA1*, which is a key gene for salt stress sensing, as well as key components of the Salt Overly Sensitive (SOS) pathway, such as *SlSOS3*, *SlTFT*, and *SlGI*. Furthermore, AMF inoculation alters the expression of genes encoding potassium (K^+) transporters and vacuolar Na^+/H^+ antiporters, contributing to enhanced Na^+ efflux and improved stem Na^+/K^+ ratio under salt stress. AMF-colonized roots exhibited elevated expression of reactive oxygen species (ROS) detoxification genes, including catalases and ascorbate peroxidases, suggesting a reinforced antioxidant defense system. Our findings indicate that AMF inoculation confers salt-stress tolerance in tomato roots by acting on multiple fronts: improving salt-stress sensing, regulating ion transport and homeostasis, and enhancing antioxidant capacity.

13 - Roots in a box: genotype specific transcriptomic profiles in barley and faba bean highlight the effect of soil volume and type in controlled condition experiments

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Root traits play a crucial role in resilience to environmental changes, and understanding how these constraints influence plant development is key for optimizing controlled-condition experiments. Within the Horizon Europe project “Root2Res: Root phenotyping and genetic improvement for rotational crops resilient to environmental change”, root traits that support yield stability and contribute to climate change mitigation under variable conditions are studied in diverse species/genotypes. In a first experiment, the impact of four pot sizes has been investigated on two barley genotypes, a landrace and a modern elite line, by combining X-ray computed tomography of roots with transcriptomic and metabolomic profiling. In parallel, the interaction between soil type and water availability in two faba bean genotypes grown in three soils under optimal, drought, and waterlogged conditions has been assessed. In barley, the landrace showed phenotypic stability across pot sizes, accumulating osmoprotectant metabolites, while the elite line was sensitive to pot size variation, with an increased root transcriptional reprogramming. In faba bean, RNA-seq analysis of roots and shoots revealed genotype-specific responses to water regimes. Further analyses using other two soil types will allow to understand how the soil type influences plant transcriptomic profiles. These findings highlight the importance of soil volume and types in plant experiments, suggesting genotype-dependent root response.

14 - Uncovering the distinct roles of 3’,5’-cAMP and 2’,3’-cAMP in plant signaling

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Cyclic mononucleotides (cNMPs), including the isomers 3',5'-cAMP and its positional isomer 2',3'-cAMP, are increasingly recognized as key signaling molecules in plants. While 3',5'-cAMP is synthesized by adenylate cyclases and hydrolyzed by specific phosphodiesterases, 2',3'-cAMP derives from RNA degradation, accumulates under stress, and may play a role in stress signaling, though its specific mechanisms of action remain unclear. This study aimed to investigate the differences between the two isomers, both systemically and locally. Proteomic analysis showed that, despite some overlap, the two isomers affect distinct sets of proteins, suggesting they play different roles in cellular processes. Non-invasive Microelectrode Ion Flux Estimation (MIFE) analysis revealed that both isomers contribute to reduce net K^+ and Ca^{2+} fluxes in Arabidopsis roots under oxidative stress, supporting their involvement in stress responses. To assess their potential interaction with cyclic nucleotide-gated channels (CNGCs), Two-Electrode Voltage Clamp experiments were performed in *Xenopus laevis* oocytes expressing AtCNGC2 or AtCNGC18, selected for their annotated interaction with 3',5'-cAMP. While 3',5'-cAMP induced K^+ and Ca^{2+} currents, 2',3'-cAMP showed no effect, indicating no specific interaction with these channels. These findings demonstrate that 3',5'- and 2',3'-cAMP act through distinct molecular pathways in plant stress signaling.

18 - Is β-Cyclocitral a signalling molecule?

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Living organisms constantly encounter potentially toxic compounds, originating both from external sources such as pollutants and from internal metabolic processes. In plants, photooxidative stress in chloroplasts triggers an oxidative burst, leading to the formation of various reactive byproducts. Among these, apocarotenoids have emerged as important signaling molecules that enhance plant stress tolerance.

This work focuses on the apocarotenoid β-Cyclocitral, which activates a SCL14-dependent detoxification pathway in response to excess light. In aqueous intracellular environments, β-Cyclocitral rapidly converts into downstream apocarotenoids, including β-Cyclocitric acid and Trimethyl cyclohexanone (TC). While β-Cyclocitric acid has shown promising roles in promoting drought tolerance, TC has yet to be detected in plant tissues and remains largely uncharacterized.

We are investigating the role of TC in plant acclimation to environmental stress, with a focus on its signaling function and molecular mechanisms. Additionally, we are exploring the transcriptional responses induced by TC and related apocarotenoids to identify both shared and distinct regulatory pathways.

Through this study, we aim to deepen our understanding of the β-Cyclocitral signaling network and its metabolites, a pathway that continues to captivate the plant science community due to its complexity and unexplored potential.

21 - SCL14 is the master regulator of the promiscuous-detoxification pathway

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Chloroplasts are essential for autotrophic growth, yet they can pose significant risks when photosynthesis is not tightly coupled with cellular demands. Under excessive light, chloroplasts produce a diverse array of molecules, including hormones and signaling compounds that mediate retrograde signaling and stress responses, as well as reactive oxygen species and lipid peroxides that can damage cellular components. We demonstrated that high light stress induces the production of the β-carotene-derived apocarotenoid β-cyclocitral, which activates an acclimation response via SCARECROW-LIKE14 (SCL14) that detoxifies peroxides and reactive carbonyl species.

Phylogenetic analysis of *A. thaliana* identified six additional SCL14-related genes (LISCLs): *SCL9*, *SCL11*, *SCL30*, *SCL31*, *SCL33*, and *SCL34*. To investigate whether these genes are functionally redundant with SCL14 or operate in distinct pathways, we isolated insertional mutants for five LISCLs, conducted transcriptomic analyses and assessed their spatiotemporal expression patterns. Additionally, we developed SCL14-GFP and SCL14-TurboID overexpressing lines to explore the cellular localization and interaction dynamics of SCL14 under stress and chemical treatments.

Preliminary findings suggest that SCL14 plays a unique role among the LISCL family members, positioning it as a central regulator of broad-spectrum detoxification. These insights open the way for future studies in crop species aimed at enhancing stress resilience.

23 - Pectin methylesterases as strategic modulators of plant–environment interactions

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The plant cell wall is a dynamic and highly regulated structure that plays a pivotal role in plant growth, development, and adaptation to environmental stress. Among the key cell wall–modifying enzymes, pectin methylesterases (PMEs) control the methylesterification status of homogalacturonan, affecting wall properties and cell signaling (Lionetti 2015; Lionetti et al. 2017). Although PME activity has been linked to stress responses (Coculo et al. 2023), the specific functions of individual isoforms are still not fully understood. This study analyzed PME gene expression in *Arabidopsis thaliana* under various environmental stress conditions, including thermal, water-related, and salt-related treatments, and in response to biotic stresses. Our results revealed the involvement of PME isoforms in specific plant's response to environmental cues. Intriguingly, PME isoforms reached the cell wall through distinct secretory routes, ranging from conventional or unconventional pathways. This diversity in secretion mechanisms may influence the localization and timing of PME activity, thereby affecting the plant's capacity to remodel its cell wall under stress. PME-mediated cell wall remodeling in plant responses to diverse environmental stresses will be discussed.

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27 - Unraveling Molecular Mechanisms of Drought Memory in Tomato Plants: Role of miR156 and Strigolactones

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The escalating climate crisis poses a major threat to crop productivity, with drought stress limiting plant growth and survival. In response, plants can develop drought memory - a phenomenon where prior stress exposure enhances responses to subsequent stress events. This memory is believed to involve complex molecular mechanisms, including hormonal signaling and transcriptional regulation.

MicroRNA156 (miR156), a highly conserved small RNA, plays a key role in coordinating both stress responses and developmental processes. Emerging evidence highlights its involvement in stress memory, interacting with the hormones abscisic acid (ABA) and strigolactones (SLs), which have been increasingly recognized for their role in drought acclimation. In particular, SLs contribute to plant adaptation to water deficit by modulating root architecture, stomatal behaviour, and hormonal crosstalk, enhancing water use efficiency and stress resilience.

We performed double-stress experiments in tomato (*Solanum lycopersicum*) using both wild-type and strigolactone-depleted genotypes, with or without treatment with the synthetic strigolactone analogue GR24. We measured stomatal conductance, photosynthetic rate, and water potential across stress and recovery phases, and analyzed MIR156A locus expression via qPCR. Expression analysis of the MIR156A locus revealed a notable memory-associated trend, supporting both its inducibility by drought stress and its dependence on the SLs. In addition, plants with intact strigolactone biosynthesis and those treated with GR24 exhibited reduced stomatal conductance during recovery, indicating a strigolactone-mediated imprinting at the physiological level. Ongoing work will assess potential epigenetic modifications at MIR156 loci to further explore mechanisms of drought memory.

28 - Plant in space, how Lactuca sativa responds to Simulated Microgravity and Hypomagnetic fields.

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Plants evolved under constant gravity and a dynamic geomagnetic field (GMF). We investigated lettuce (Lactuca sativa L.) responses to hypomagnetic fields (hMF) and simulated microgravity (s-µg) using a Random Positioning Machine and a Triaxial Helmholtz Coil System. Morphological, biochemical, and transcriptomic analyses revealed significant gene regulatory changes impacting energy metabolism, ion homeostasis, and stress responses. hMF induced a targeted stress response, altering root architecture, reducing chlorophyll, and modulating reactive oxygen species (ROS), reflecting resource conservation. Conversely, s-µg triggered a systemic reorganization of cellular architecture, disrupting protein processing, organelle positioning, and photosynthetic efficiency. Differential ROS and non-photochemical quenching (NPQ) responses highlighted unique adaptive challenges. Despite these differences, shared gene expression patterns revealed core adaptive mechanisms, showcasing lettuce's ability to activate conserved pathways under diverse stressors. hMF upregulated genes related to stress and energy management, while downregulating photosynthesis related genes. s-µg downregulated genes related to cellular functions. Notably, s-µg induced a complex reprogramming, characterized by selective metabolic activation and broad suppression of essential processes. Our findings provide critical insights into plant adaptation to altered environments, suggesting plant resilience this contexts.

31 - Argonaute3 is involved in hypoxia tolerance in Arabidopsis thaliana

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In plants, submergence often leads to hypoxia, a condition where limited oxygen availability impairs mitochondrial respiration and disrupts cellular energy balance. This stress triggers major changes in gene expression to support adaptive responses. Although RNA interference (RNAi) plays a crucial role in gene regulation, its involvement in hypoxia remains largely understudied. In this work, we investigate the role of ARGONAUTE 3 (AGO3) in the hypoxic response of *Arabidopsis thaliana*. Our results show that *AGO3* expression is induced under low-oxygen conditions through a mechanism downstream of the N-degron pathway. To further explore the biological function of AGO, we performed AGO3 immunoprecipitation followed by small RNA sequencing. This approach uncovered AGO3-associated RNA species and gave a hint on its physiological role. Collectively, our work highlights AGO3 as a potential mediator between RNAi and the plant hypoxic response, offering new insights into stress-regulated RNA signaling mechanisms. These findings suggest that AGO3 may serve as a molecular link between RNAi pathways and the hypoxic response, shedding light on previously uncharacterized layers of post-transcriptional regulation during environmental stress adaptation.

32 - APPLE LEAF REACTION TO FREEZING STRESS IS MITIGATED BY INOCULATIONS WITH COLD-TOLERANT BACTERIA

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Keywords: freezing stress, cold-tolerant bacterial endophytes, apple plants

Climate warming is responsible for mild winters and warm springs, causing premature plant development and increasing the risk of exposure of vulnerable plant tissues to spring frost. Cold stress triggers changes in membrane fluidity and accumulation of reactive oxygen species (ROS). Cold-tolerant bacteria colonize wild Rosaceous plants in alpine regions, but limited information is available on the beneficial plant-microbe interactions in the mitigation of freezing stress. The aim of this project was to characterize the efficacy and mode of action of cold-tolerant endophytic bacteria isolated from alpine plants in mitigating freezing stress in apple tissues. Three cold-tolerant bacterial isolates (two *Pseudomonas* spp. and one *Duganella* spp.) can decrease electrolyte leakage in strawberry seedlings upon freezing stress. Apple seedlings (cultivar Golden Delicious) were grown under controlled conditions, treated (bacterium-inoculated) or not (mock-inoculated) with cold-tolerant bacteria, and exposed to freezing stress. Bacterial inoculation decreased ROS content and malondialdehyde (MDA) in apple leaves. Moreover, transcriptomic analysis revealed the upregulation of genes related to biological processes of carbohydrate metabolism, stress response, and signal transduction in bacterium-inoculated plants after freezing stress. Thus, cold-tolerant bacteria isolated from alpine Rosaceae plants can mitigate freezing stress in apple plants.

This project has received funding from the Fondazione CARITRO, Cassa di Risparmio di Trento e Rovereto (project MITICLIMA).

37 - Unveiling capacity and consequences of isoprene perception by phenotypic and physiological characterization of stressed isoprene-receiving plants.

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Plants use volatile organic compounds (VOCs) to communicate with each other. VOCs sent by emitters may influence gene regulation, metabolism, phenotype, responses to stress, and behavioral choices of receiving plants. Our research aims to study if and how isoprene inform non-emitting receivers of imminent drought or heat stress. We are thus characterizing phenotypical and physiological features of isoprene receivers after stress. A first experiment was carried out on *Arabidopsis thaliana* wild-types that do not emit isoprene (WT), and on genetically modified isoprene-emitting plants (ISPS). Some WT plants were fumigated in a jar for 4 hours with 1 µl of isoprene (WTf). WT, WTf and ISPS plants were then subjected to heat stress for 18h at 37°C. Petiole hyponasty (upward movement of leaves, measured using Image J) was chosen as significant index of stress response (phenotypic trait). After stress exposure, hyponasty resulted significantly lower in both ISPS and WTf plants compared to WT plants. ISPS plants, however, showed significantly lower maximal and actual fluorescence yield (Fv/Fm and PhiPSII) than WT and WTf. Results therefore indicate that plants receiving exogenous isoprene can sense the volatile. Isoprene sensing alters the phenotypic response to the stress but is not always related to improved resistance to stress. Preliminary results collected with *Nicotiana tabacum* as a second model species, confirm the capacity of isoprene sensing by non-emitting receivers, but also show improved resistance to stress in these plants. Overall, these results will hopefully contribute to unravel how VOCs control plant-plant communication and activation of stress responses.

Keywords
Isoprene; hyponasty; stress response; photosynthesis.

40 - Sustaining seagrass restoration: long-term preservation of Posidonia oceanica seeds and seedlings

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Seagrass restoration efforts using *Posidonia oceanica* traditionally rely on lateral cuttings, a costly and labor-intensive method that damages parent meadows and limits genetic diversity. Seedling-based propagation presents a viable alternative which ensures genetic variability but is constrained by the unpredictability of seed stranding events and the short window for seed collection. To address this limitation, we tested methods for long-term seed and seedling storage, aiming to extend transplanting opportunities beyond natural germination cycles. We evaluated the effects of light or dark conditions, density, and seedling age on viability during storage at 4°C for six months. Our results demonstrate that light is essential for preserving viability, as all dark-stored samples died post-storage. Older seedlings (1-2 months old) exhibited higher survival rates (70-90%) compared to freshly collected seeds (40%). Seedling density did not significantly affect viability, simplifying large-scale storage. Attempts to induce dormancy with ABA or paclobutrazol were unsuccessful. This study provides the first successful protocol for seed and seedling storage, enabling year-round planning of seagrass restoration projects and enhancing their feasibility and efficiency.

41 - Divergent roles of RBOHD, AtCuAOβ, and GLR3.3 in systemic wound signaling

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Reactive oxygen species (ROS) function as crucial second messengers in long-distance wound responses, coordinating systemic stomatal closure. In this study, we dissect the relative contributions of two ROS-generating enzymes—the plasma membrane NADPH oxidase RBOHD and the apoplastic amine oxidase AtCuAOβ—and the glutamate receptor-like ion channel GLR3.3, to both local (wounded leaf) and systemic (distal leaf) ROS accumulation and Ca^{2+} dynamics in Arabidopsis. In wild-type (WT) seedlings, leaf wounding triggers rapid ROS accumulation both within guard cells—detected by confocal microscopy using CM-H_2DCFDA—and leaf tissues, observed *via* whole-seedling fluorescence imaging. They also exhibit a fast-propagating Ca^{2+} wave, detected using the red-shifted R-GECO1 indicator. *Atcuaoβ* mutants fail to exhibit any detectable ROS increase, either locally or distally, yet retain a WT-like Ca^{2+} wave, indicating an essential role for AtCuAOβ in both local and systemic ROS production. *rbohd* mutants show WT-like levels of local ROS but reduced distal ROS accumulation and delayed Ca^{2+} wave propagation, highlighting the involvement of RBOHD mainly in long-distance signaling. *glr3.3* mutants exhibit attenuated ROS accumulation in both local and distal tissues, underscoring the importance of GLR3.3 in Ca^{2+}-dependent amplification of ROS signals. Overall, these findings provide new insights into the role of ROS–Ca^{2+} crosstalk in the coordination of local and systemic wound signaling.

42 - The role of HPCA1 in ROS perception and long-distance signal propagation after wounding in A. thaliana

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Plant perception of abiotic stress can initiate long-distance signaling involving rapid systemic waves, that are hydraulic, electric, reactive oxygen species (ROS) and Ca^{2+} waves, triggering acclimation responses. Recent studies shed light on how apoplastic ROS accumulation is sensed along the signal path, altering cytosolic Ca^{2+} levels and involving membrane proteins. In this study, we investigate the role of the H_2O_2 receptor HPCA1 (H_2O_2-INDUCED Ca^{2+} INCREASES 1) in systemic ROS signaling in Arabidopsis. Analyses of stomatal movements in *hpca1* revealed that the mutant failed to respond to various stress conditions (leaf/root wounding or H_2O_2 treatment), with no stomatal closure observed, and displayed a partially closure impairment after ABA treatment, unlike the WT response. Stomatal ROS accumulation after wounding was visualized using the ROS indicator CM-H_2DCFDA, which showed a reduction of ROS accumulation within *hpca1* guard cells, compared to WT. Finally, to investigate whether HPCA1 is involved in ROS signal propagation, the analysis of ROS accumulation in whole seedlings after leaf or root wounding via CM-H_2DCFDA was performed. *hpca1* mutants showed reduced ROS accumulation compared to WT in both local and distal cotyledons, with a stronger impairment in the distal one, suggesting defective systemic signal propagation. Overall, evidence suggests that HPCA1 is a key regulator of ROS signaling and highlights its essential role in mediating systemic stress responses.

46 - Root traits control methane emissions from rice

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Flooded paddies are an important source of greenhouse gases. During soil flooding, anaerobic microorganisms produce CH_4, which accumulates in the flooded soil due to limited gas diffusion. Rice roots form aerenchyma to facilitate intra-tissue O_2 diffusion to the growing root tips. At the tip, molecular O_2 diffuses into the rhizosphere supporting CH_4 oxidation to CO_2. However, CH_4 also penetrates the root tissues and diffuses upward through the aerenchyma before being emitted into the atmosphere. The mechanisms of CH_4 diffusion from the rhizosphere to and along the roots and the root characteristics influencing such gas diffusion are largely unknown.

We cultivated rice in a controlled-temperature chamber in different hydroponic treatments (aerated, deoxygenated, low-nutrients) to prompt changes in root morphology and anatomy. Furthermore, both roots and shoots were cut at different heights to identify the prevailing pathways of CH_4 release from the plants. CH_4 and CO_2 fluxes were measured in a static chamber where the plants were in a solution with 10% CH_4 and the shoots in air. Roots were collected to evaluate their architecture, anatomy, and the deposition of suberin and lignin.

Plants grown in different solutions showed variation in root aerenchyma formation and the development of a barrier to radial oxygen loss, resulting in limited CH_4 emissions. Root manipulation experiments revealed that decreases in root area led to diminished CH_4 emissions. Removing shoots or closing stomata did not significantly affect CH_4 emissions but decreased CO_2 uptake. These findings suggest that roots, rather than shoots, are the primary tissues regulating CH_4 diffusion in rice.

48 - Elucidating Differential Heat Stress Responses in Tomato Genotypes Through Integrated Physiological and Genomic Analyses

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As tomato is a major crop in Mediterranean regions, it has become essential to identify the factors influencing its productivity under stressful environmental conditions. This study examined two thermotolerant tomato varieties (LA3120 and E42) grown in a controlled environment at different temperatures, in order to evaluate their biological responses to increased heat levels. The results revealed that thermal stress affected gas exchange and chlorophyll fluorescence measurements differently in the two varieties, as well as the accumulation of secondary compounds. Specifically, E42 effectively controlled photosynthesis during thermal stress by modifying the rate of electron transport, while LA3120 increased flavonoid levels by altering secondary metabolic pathways under heat stress. Genetic profiling data obtained through GBS (genotyping by sequencing) analysis allowed us to investigate genomic variation between the two varieties. High and moderate polymorphisms were particularly found in genes involved in photosynthetic mechanisms that could be involved in controlling heat stress. This approach identified 22 potential genes, 13 of which exhibited mutations unique to E42, and seven of which were specific to LA3120. These findings expand our understanding of the biological processes underlying thermal stress responses in tomatoes. They also demonstrate how these tomato varieties can serve as valuable sources of traits associated with adaptation to environmental stress.

54 - Variability in salt stress tolerance of wild Brassica rapa seedlings originating from different Mediterranean areas

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Salinity is one of the main abiotic stress factors for plants that is steadily increasing at the global level. This study investigated the effect of salt stress (NaCl 50 and 150 mM) on three wild genotypes of *Brassica rapa* L, originating from Algeria, Italy, and France (*BR-A-CHLE-W*; *BR-I-GRAT-W; BR-F-FOUG-W*), whose seeds were obtained through the activities of the BrasExplor project (PRIMA 2019). The aim was to highlight potential differences in the response of functional, physiological and morphological traits. After 21 days of treatment, the following parameters were analysed: chlorophyll fluorescence (Fv/Fm), relative membrane permeability (RMP), biomass, shoot length and leaf area. CHLE showed good tolerance at 50 mM, though significant reductions in leaf area and shoot length at 150 mM; GRAT was sensitive already at 50 mM; FOUG exhibited atypical responses, with partial recovery at 150 mM. In all genotypes, Fv/Fm progressively decreased, while RMP increased, with FOUG displaying high values even under control conditions. Biomass responses were variable. These results highlight considerable variability in the response to salt stress among the analysed genotypes. In the context of increasing soil salinization in agricultural areas worldwide, studying such different responses represents an important step toward the possible future selection of tolerant lines, offering new perspectives for the genetic improvement of *Brassica rapa*.

55 - Mining for salt tolerance indicators in the genus Vigna

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The amount of salt affected arable land is increasing rapidly, threatening food security on a global scale. Wild relatives of staple crop provide information regarding abiotic stress tolerance and genetic material that may be used to increase resistance through artificial breeding. In the present study, physiological, genomic and metabolomic approaches were used to characterize the response of five species of the genus *Vigna* to a 3-week treatment with 0, 100, 200 and 300 mM NaCl: domesticated and wild accessions of *V. unguiculata*, and purportedly salt tolerant *V. radiata*, *V. luteola* and *V. vexillata*. Biomass, leaf gas exchange, fluorescence and chlorophyll concentrations were all dramatically reduced in domesticated *V. unguiculata* at even the lowest salt concentration, indicating this crop's increased sensitivity to elevated salt relative to its wild relatives. Biochemical and genetic analyses of tissue samples are underway to examine salt tolerance mechanisms that involve the production of protective secondary metabolites through GC-MS of metabolomic profiles and the relative expression levels of transporters (ex. SOS1-3, NHX1,2, and HKT) that are known to regulate inter-tissue, and inter- and intracellular ion movement through qRT-PCR. Combining these results with available phylogenetic data will guide the selection of closely related wild accessions that may be used to improve salt tolerance in the domesticated lines of species within the genus *Vigna*.

57 - Hydrochar as a soil amendment in sunflower: linking physiological responses and molecular mechanisms

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Hydrochar (HC) from waste biomass is a sustainable strategy to improve soil quality and enhance plant drought tolerance, though its molecular effects remain largely unexplored. In this study, HC from garden waste was tested in *Helianthus annuus* L. under well-watered and drought conditions through physiological, molecular, and soil analyses. HC significantly supported plant growth, especially under drought, by improving soil water retention and enabling gradual nutrient release. These effects enhanced CO_2 assimilation, made the leaf turgor loss point more negative, and increased biomass vs. controls. Besides improving soil, HC induced strong transcriptional reprogramming in both primary and secondary metabolism. This included the maintenance of photosynthesis and core metabolism through the modulation of gene networks tied to redox balance and cellular homeostasis. Specific gene modules correlated with water use efficiency, also identifying *hub* genes. The study offers a comprehensive view of HC's role in drought resilience and its value for sustainable soil and crop management.

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59 - Comparative analysis of Glyoxalase I gene expression in modern and ancient durum wheat (Triticum durum Desf.) genotypes under water stress at both vegetative and reproductive growth stages

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Glyoxalase I (GLYI), a key enzyme in detoxifying the glycating agent methylglyoxal, plays a crucial role in plant abiotic stress tolerance. Recently, the full *GLYI* gene family was identified in durum wheat (DW) showing an upregulation by salt and osmotic stresses in early etiolated seedlings.

Here, we investigated the expression of five *GLYI* genes in modern (Svevo and Ofanto) and ancient (Senatore Cappelli) DW genotypes grown under control conditions and moderate water stress (50% soil capacity), applied at stem elongation (S1) or flowering (S2). Leaf samples were collected after 10–15 days of stress, and at flowering in S1-plants that had been re-watered (R) after stress.

Water stress triggered distinct *GLYI* gene expression profiles depending on growth stage and genotype. Notably, Svevo showed upregulation by stress of all genes at both stages up to 4.5-, 22- and 30-fold in S1-, S2- and R-plants, respectively. In contrast, Ofanto exhibited activation of only three genes at flowering, with up to a 3-fold increase in R-plants. In Cappelli, a slight (1.6-fold) activation of two genes was observed only in S2-plants.

Overall, these findings enhance our understanding of GLYI function in plants, strengthening our previous evidence of its key role in water stress tolerance in DW. They also reveal constitutive differences between ancient and modern wheat varieties, highlighting the superior stress-responsive capacity of modern genotypes—particularly Svevo—compared to the ancient Cappelli.

61 - Phytoextraction potential of Dittrichia viscosa in antimony-contaminated environments

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Trace metals use in human activities leads to their accumulation in wastewater and sewage sludge. At the Prato wastewater treatment plant (WWTP), managed by GIDA SpA, sludge may contain metal(loids) like antimony (Sb), mainly from local textile industries. While sludge is typically incinerated, phytoextraction offers a low-cost alternative for remediation. Metallophytes, including excluders and (hyper)accumulators, are valuable tools for phytostabilization and phytoextraction of toxic metals.
After screening spontaneous species at the Sb-contaminated Su Suergiu mine (Villasalto, Sardinia), *Dittrichia viscosa* was selected due to high Sb levels in its shoots, suggesting it as an indicator species. This study assessed Sb tolerance and accumulation in three different *D. viscosa* populations (both metallicolous and non-metallicolous) grown hydroponically and exposed to increasing Sb concentrations for two weeks. The non-metallicolous population showed reduced biomass and root length, along with altered physiological traits and pigment content.
Antimony was accumulated more in the roots than in the shoots, with significant higher levels in both metallicolous populations Although *D. viscosa* proved not to be a hyperaccumulator, it may serve as a useful indicator species. Such preliminary studies are key for selecting suitable candidates for Sb phytoextraction. In the near future plants will be tested directly in sewage sludge from the GIDA WWTP to confirm their Sb tolerance and accumulation capacity.

66 - Long-term plant memory toward repeated drought stress assessed by grapevines physio-anatomical responses

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Recently, investigation of plant memory phenomena in response to abiotic stresses has become a subject of interest due to increasingly intense and frequent drought events. It has been suggested that plants can “remember” past stress occurrence. Grapevine, one of the most economically important crops worldwide, is particularly vulnerable to environmental stressors impacting growth and fruit quality. Although physio-anatomical adjustments were observed in grapevines after recurrent drought exposures in the same growing season, little is known about persistent plant memory occurring years after recurrent drought. To unravel this mechanism, plants of *Vitis vinifera* cv Nebbiolo (CVT 185) grafted onto the SO4 rootstock were subjected to a priming treatment consisting in repeated cycles of mild drought and recovery imposed over four years. Then, primed plants were exposed to severe drought, concomitantly with a group of unprimed vines in the fifth year. Hydraulic and anatomical measurements performed on current year sprouts, stem at 5 cm above grafting point and rootstock revealed no significant differences between drought-primed and unprimed plants, but they showed changes depending on the applied drought intensity (mild *vs.* severe). In conclusion, drought priming treatment has no effect on the studied plant characteristics, while drought intensity turns out to be the main factor modulating physio-anatomical features of grapevine.

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68 - Carbon reserves in old Abies alba trees as a function of tree-rings growth

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The fate of forests is threatened by changes in environmental conditions. In our work we investigate the relationship between anatomy and physiological mechanisms involved in tree acclimation responses. Dendrochronology is a powerful tool to assess eco-physiological drivers of tree growth at interannual resolution and the potential to invest in an adequate pool of non-structural carbohydrates (NSC) should be considered.

Trees archive their responses to stressors within their datable growth layers; we reconstructed xylem anatomical traits (i.e. ring width) and the ring-age-related distribution of NSC in old trees *Abies alba*. This could be used as a sensitive and effective diagnostic tool to predict which trees are more prone to suffer from extreme climate events, and such as drought-induced die-off.

Our data suggested that NSC concentration is correlated with annual growth and wood density. Moreover, we postulated that the ratio between soluble NSC and starch was modulated by water scarcity/availability, depicted by ring thickness.

We hypothesized that trees rely entirely on the remobilization of reserves stored during the previous growing season to support stem radial growth, yet this impacts the capacity of resistance to drought events. Future investigation calls for a thorough evaluation of different *Abies alba* populations for a rigorous evaluation of the link among genotypes, phenotypes, physiological traits, and climatic drivers constraining tree growth across time and environments.

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75 - Seeing the unseen: microplastics effects on tomato hydraulics and non-structural carbohydrates

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Microplastic pollution is emerging as a serious threat to plant health, yet its impact on hydraulic and metabolic functions remains unclear. We investigated how different concentrations of 1 μm polystyrene (PS) microplastics affect leaf and whole plant hydraulic conductance, water status, and carbon allocation in *Solanum lycopersicum* L. plants. Exposure to PS significantly reduced hydraulic conductance and gas exchange, leading to stunted growth and altered accumulation of non-structural carbohydrates in both roots and leaves, with more severe effects at higher concentrations. Treated plants also exhibited thinner leaves, with more negative water potential and lower leaf mass per area. A non-destructive optical method was used to measure leaf vein vulnerability to xylem embolism, while SEM and Raman spectroscopy analysis are ongoing to pinpoint PS accumulation sites within tissues. Our results show that microplastics disrupt plant functions by impairing water transport and altering carbon allocation, reducing root storage while increasing leaf accumulation, ultimately threatening plant health and crop productivity.

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78 - Mitochondrial Calcium Signaling in Plants: Insights into Stress Response Mechanisms

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depends on the rapid detection of stress and the activation of appropriate responses. While the plasma membrane is often seen as the primary sensor, mitochondria, being highly stress-sensitive, may serve as early sensors via retrograde signaling, which communicates organelle status to the nucleus.

In Arabidopsis, mitochondria-derived ROS are known to trigger retrograde signaling, but Ca^{2+} ions may also play a role. The mitochondrial Ca^{2+} uniporter complex (MCU and MICU) regulates Ca^{2+} uptake: MCU remains closed at low cytosolic Ca^{2+} levels but opens when MICU detects elevated Ca^{2+} concentration.

To explore mitochondrial Ca^{2+} dynamics under environmental stress, we developed a dual-sensor *Arabidopsis* line expressing a red-shifted mitochondrial and a green-shifted cytosolic Ca^{2+} indicator, enabling real-time, in vivo imaging.

Focusing on hypoxia, which impairs mitochondrial respiration, we observed rapid increases in both cytosolic and mitochondrial Ca^{2+} concentration upon oxygen deprivation. Interestingly, mitochondria acted as a Ca^{2+} sink rather than a source, contrary to previous suggestions. To assess the role of MICU in regulating mitochondrial Ca^{2+} dynamics during hypoxia, we also generated *micu* mutants expressing different mitochondrial Ca^{2+} indicators. Analyses are currently ongoing.

79 - CYTOKININS AND ROS REGULATE THE ROOT MERISTEM SIZE AND ITS MAINTENANCE UNDER SALT STRESS IN RICE.

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The root is essential for plant nutrition, anchoring, and sensing soil born stresses. Its structure, shaped by genetics and environment, is dynamic and adapts during growth—a trait known as plasticity—regulated by the root apical meristem (RAM). In Arabidopsis, RAM activity is influenced by hormones and ROS. Salinity hinders root growth, reduces root hairs, and alters architecture, threatening productivity. Rice, often grown in salinity-prone coastal areas, is especially vulnerable. Understanding how RAM is maintained under salt stress is crucial. Given the conservation between Arabidopsis and rice RAM, we explored shared mechanisms. Imaging rice seminal roots revealed that meristem size and transition zone (TZ) positioning are affected by cytokinins, ROS, and salinity. We observed similar H_2O_2 and O_2^- distributions and identified rice genes homologous to Arabidopsis ones (PINs, RRs, GH3s) with expression changes under cytokinin and H_2O_2 treatment. Comparing salt-sensitive and tolerant rice varieties showed that tolerant ones better maintain RAM size by modulating hormone responses. These findings highlight conserved mechanisms in RAM regulation and offer insights into rice root plasticity under salinity—key to improving crop resilience.

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80 - Beneficial effects of irrigation with Coffee Silverskin Solutions on growth and metabolism of Brassica oleracea plants.

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Coffee Silverskin (CS) is the only by-product generated during coffee roasting. Every year, several million cubic meters of CS are produced and stored. In this study, the authors assess the effects of a treatment using a solution formulated from CS on the growth and cultivation of broccoli plants (*Brassica oleracea*). Broccoli plants were cultivated for 4 weeks and periodically watered or sprayed with the CS solution (CSS) at different concentration (2% or 4%). Physiological and morphological changes in the plants under the experimental conditions were evaluated. Treatment with the CSS demonstrated a stimulatory effect, particularly in plants watered with the 4% solution. The watered plants exhibited improved and more vigorous growth, as evidenced by increased shoot height, number of leaves, leaf area, and weight; the ratio of net photosynthesis to stomatal conductance indicated enhanced gas exchange in these plants, just as a reduction in NPQ highlights a more efficient photosynthesis. Protein and polyphenol contents were also higher in irrigated plants, and auxin levels were significantly greater in these plants compared to the control group. In conclusion, the irrigation with CSS enhances the phenotypic traits and biochemical quality of *Brassica oleracea*, suggesting its potential as a beneficial growth stimulant, while foliar spraying with CS solution did not result in significant improvements of plants.

83 - Application of exogenous VOCs on tomato plants (Solanum lycopersicum cv. Micro-Tom) to prime tolerance to water stress

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Volatile organic compounds (VOCs) emitted by plants play a dual role as potent defensive molecules and efficient mediators of communication among plants and with other organisms. Priming occurs when healthy plants exposed to VOCs emitted by neighbour plants experiencing biotic or abiotic stress perceive them as an “alarm signal” and rapidly activate physiological, and biochemical responses able to mitigate the forthcoming stress. Water deficit is arguably the main abiotic stress negatively impacting plant growth and overall productivity in both natural ecosystems and agroecosystems. In this study, we employed tomato plants (*Solanum lycopersicum* cv Micro Tom) to evaluate the priming efficacy of three VOCs (β ionone, nonanal and methyl jasmonate (MeJA)) against water stress. Plants were placed inside a glass chamber and exposed for 24 h to an aliquot of either one of the three VOCs. Thereafter, half of the individuals of each group (primed and non primed) were exposed to water stress. Gas exchange analyses revealed that β ionone and nonanal significantly increased stomatal conductance in non stressed plants, but this effect disappeared following drought imposition and did not translate into improved water use efficiency. MeJA induced no significant changes, likely due to its role as an endogenous intermediate in multiple metabolic pathways. VOC exposure altered the volatile emission profile of treated plants, a feature warranting further investigation. In the Micro Tom model, β ionone and nonanal are sensed by receiving non-stressed plants, but none of the tested VOCs effectively prime resistance of tomato plants against water stress.

84 - GREEN AND FAR-RED LIGHTS: KEY ACCLIMATION DRIVERS THAT BOOST PLANT GROWTH

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The use of LED technology in controlled environment agriculture has enabled precise control over the light spectra provided to plants. Initially, lighting strategies focused mainly on red and blue (RB) wavelengths, while green (G) and far-red (Fr) light were excluded to reduce energy consumption. However, recent research has shown that G and Fr light contribute significantly to plant development, morphology, and photosynthesis.

This study examined the effects of supplementing traditional RB lighting with G and Fr light on *Lactuca sativa*, focusing on both morphological and physiological acclimation. Various light treatments were tested under both continuous and discontinuous spectra. Photosynthetic performance was assessed using chlorophyll fluorescence and gas exchange techniques, while biochemical analyses provided insights into the composition of the photosynthetic apparatus. Morphological traits were studied through relative growth rate index (RGR).

After two weeks of exposure, plants grown under light that included G and Fr wavelengths accumulated over 30% more biomass than those grown under RB light alone. Interestingly, RB light triggered a typical high-light-like response—increasing photosynthesis and electron transport—but also resulted in thicker leaves and a lower shoot/root ratio, increasing the metabolic cost of growth. In contrast, G and Fr light induced a low-light-like acclimation, promoting leaf expansion and ultimately enhancing RGR.

Our findings suggest that incorporating G and Fr wavelengths into LED lighting systems can improve plant growth efficiency, helping to meet the demands of modern indoor agriculture.

NODES Spoke 2, PoC PFS and GRINS PE0000018 - CUP D13C22002160001) are acknowledged

86 - Exogenous MicroRNA extraction in soil-grown Arabidopsis thaliana

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MicroRNAs are non-coding RNAs playing critical roles in gene regulation within organisms, but are increasingly recognized as signalling molecules in the plant-environment interface. When *Arabidopsis thaliana* plants grow in a hydroponic system, microRNAs can be found in the external growth medium. The expression levels of these microRNAs can influence target gene expression in neighbouring plants sharing the same medium. Isolating plant-derived nucleic acids from the environment without contamination by root parts is necessary to describe molecular communication from plants to their surrounding environment.

We developed a methodology for the extraction of environmental RNA, with a specific focus on microRNAs, from the substrate surrounding *Arabidopsis* plants. Plants were grown in soil in a confined environment designed to prevent root penetration into the sampling area. RNA was extracted from the water supplied to the plants, collected after percolation through the substrate. Then, we evaluated the effect of two different substrates on nucleic acids detectability: peaty soil and clayey soil, which can influence nucleic acid binding, degradation, or transport.

Our approach aims to avoid root contamination, allowing for a clear assessment of plant-released nucleic acids, and to determine the contribution of the growing medium to the overall detectability of environmental RNAs.

87 - Hypericum perforatum and Gentiana lutea plant extracts: phytochemical profile and potential biological activities of two native plants from Pollino National Park

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Plants synthetize a wide variety of secondary metabolites known for their physiological and ecological effects. Within the ONFOODS project, *H. perforatum* and *G. lutea*, two native plants from the protected area of Pollino National Park, were investigated for their potential biological properties.

H. perforatum and *G. lutea* leaves, flowers, and fruits were collected in Calabria in different ecosystems. Samples, properly dried and pulverized, were extracted through maceration (EtOH 80%). Then, the obtained suspension was filtered and concentrated at a rotary evaporator (37 °C). The phytochemical profile was investigated by means of TLC, HPLC and phenolics content, while the antioxidant activity was evaluated through ABTS test and TLC-bioautography-DPPH. In addition, the anti-adipogenic potential properties were assessed in 3T3-L1 cell line.

The different chromatographic methods revealed qualitative and quantitative differences among extracts collected in different ecosystems. As regards the phenolic content and the antioxidant potential, *G. lutea* showed a comparable trend for each analyzed tissue, while in *H. perforatum* samples, leaves, followed by flowers and fruits, showed the best activity.

The ability to inhibit the formation of lipid droplets was also determined: the highest sub-cytotoxic doses (MTT test), were tested through Red Oil O assay. *H. perforatum* leaf extract (40 µg/mL) showed remarkable activity, suggesting it could be considered a potential anti-obesity agent.

89 - Microplastics in the soil at sub-toxic concentrations cause metabolic changes decreasing fungal pathogen susceptibility in Arabidopsis thaliana

Marco Dainelli [1] *- Costanza Cicchi* [2] *- Ivan Baccelli* [3] *- Stéphanie Boutet* [4] *- Ilaria Colzi* [1] *- Andrea Coppi* [1] *- Simone Luti* [3] *- Sara Pignattelli* [5] *- Susanna Pollastri* [3] *- Francesco Loreto* [3] *- Luigia Pazzagli* [2] *- Massimiliano Corso* [4] *- Cristina Gonnelli* [1]

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To unravel the complex interactions between microplastics (MPs), plants, and pathogens, *Arabidopsis thaliana* plants were grown for three weeks in soils containing polyethylene terephthalate (PET) or polyvinyl chloride (PVC) MPs (0.2% and 0.5% w/w), and leaves were then exposed to the PAMP (Pathogen-Associated Molecular Pattern) protein cerato-platanin (CP) or to *Botrytis cinerea* conidia. PET clearly caused a stimulation of stomatal conductance, and PVC decreased aboveground biomass of *A. thaliana* plants. PVC (0.2%) triggered a primed state in *A. thaliana*, enhancing its response to *B. cinerea* infection and cerato-platanin. This was demonstrated by decreased lesion size, enhanced ROS generation, elevated camalexin synthesis following PAMP elicitation, and increased levels of defensive isothiocyanate and phenylpropanoid metabolites. Our results indicate that MPs also affect soil structure, ionome balance, and specialized metabolite accumulation. However, MPs did not provide an unambiguous response, underscoring challenges in formulating a model of plant response to MPs when exposed to pathogens.

93 - Bridging model systems and crop improvement: expression of the Arabidopsis Salt Tolerance-Related Protein (STRP) enhances cold tolerance in tomato

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Abiotic stresses severely affect plant development, metabolism, and productivity, posing a major threat to global food security. Defense proteins are key components of plant adaptive responses, and their functional characterization in model species can guide the development of stress-resilient crops. The Salt Tolerance Related Protein (STRP), a hydrophilic and intrinsically disordered protein from *Arabidopsis thaliana*, has been functionally characterized for its ability to confer tolerance to both salt and cold stress. In this study, we evaluated the potential of STRP to enhance cold tolerance in *Solanum lycopersicum* by generating stable transgenic lines constitutively expressing *STRP* fused to *Yellow Fluorescent Protein* (*YFP*). *STRP-YFP* expression led to more efficient seed germination and promoted root elongation in early seedling development. Upon exposure to cold stress, transgenic plants showed reduced cold-induced damage, including decreased ion leakage and lower chlorophyll degradation compared to *wild type*. Moreover, biochemical analyses conducted under cold stress conditions suggest that STRP retains its typical functional dynamics in tomato, including its stress-induced increase and nuclear localization. These results demonstrate that *STRP* expression improves cold stress tolerance in tomato, highlighting the value of translating functional insights from model species to crops for climate-resilient agriculture.

94 - cAMP as a Key Regulator of Basal Thermotolerance: Insights from Redox and Transcriptomic Analyses in Arabidopsis

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Global warming led to significant changes in seasonal temperatures, resulting in more frequent heat waves. Plants perceive high temperatures as heat stress (HS) and activate defense mechanisms to develop thermotolerance. Under stress conditions, redox homeostasis, maintained by balancing the production and removal of reactive oxygen species (ROS), can be disturbed. This activates redox-dependent signaling pathways involved in the heat stress response (HSR). This study explores the link between redox signaling and the second messenger cAMP in the HSR of *Arabidopsis thaliana*. We analyzed two transgenic lines (cAS plants) that express a cAMP-sponge construct, sequestering intracellular cAMP. Fifteen-day-old wild-type and cAS plants were exposed to 45°C for 2 hours and then returned to 22°C for 3 or 48 hours to assess recovery. The cAS plants showed increased sensitivity to HS, evidenced by reduced fresh weight, chlorotic leaves, and a lower content of photosynthetic pigments, indicators of impaired recovery after 48 hours. Consistently, the chloroplast ultrastructure in cAS plants was more severely affected. Also, these plants exhibited an altered redox-dependent response during recovery, resulting in ROS accumulation and oxidative damage. Transcriptomic analysis revealed a reduction in the expression of protein-folding genes in the cAS plants, which may contribute to the observed oxidative stress. These findings suggest that cAMP plays a crucial role in basal thermotolerance.

95 - The role of non-structural carbohydrate availability for turgor maintenance under drought: implications for xylem hydraulic safety

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Drought-induced depletion of non-structural carbohydrates (NSC) has been reported to increase xylem hydraulic vulnerability, but conclusive evidence supporting a mechanistic link between the two processes is missing. Given that NSC depletion can impair osmoregulation, we hypothesised that earlier turgor loss and consequent plasmolysis of vessel-associated parenchyma cells (VAC) during drought in NSC-depleted plants could facilitate gas propagation from VAC to vessels and earlier xylem embolism formation.

Plasmolysis of VAC was induced in *Populus nigra* stems by applying osmotic treatments, and determined an increase in xylem vulnerability to embolism, suggesting a relation between the two processes. In addition, potted saplings were depleted in stem NSC by prolonged (50 days-long) shading (S) and then subjected to drought, in order to verify whether a less negative turgor loss point (TLP) in these samples leads to higher vulnerability to xylem embolism compared to control light-exposed plants (L). Xylem vulnerability to embolism of S and L plants was compared with measurements of stem TLP and NSC (sugars and starch) content, as well as proline and inorganic ions content, in order to quantify the relative contribution of sugars and other solutes to osmoregulation/protection under NSC limitation and the related impact on xylem vulnerability.

Our study offers new insights into the role of NSC reserves in shaping plant hydraulic behaviour and driving plant survival under drought.

97 - Plant-based biostimulant effects on leaf secondary metabolites under abiotic stress conditions in grapevine

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This research aimed to evaluate the effects of a biostimulant distribution on the biochemical response of *Vitis vinifera* cv Merlot under drought stress, focusing on the accumulation of leaf phenolic compounds involved in defence and stress mitigation.

Total leaf polyphenolic index was quantified using the Folin-Ciocalteu method. Individual polyphenols were separated through high-performance liquid chromatography coupled with a diode-array detector (HPLC-DAD), which allowed the quantification of flavonols, hydroxycinnamoyltartaric esters and low molecular weight flavan-3-ols.

Results show that the biostimulant application increased the concentration of total polyphenols in the leaves of intensively water-stressed vines, mainly throughout the modulation of flavonol and low molecular weight flavan-3-ol concentrations.

The biostimulant application boosted plant primary metabolism: increased glucose availability, presumably resulted in a variation of the flavonol profile that was richer in flavonol-glucosyl derivatives respect to that detected in non-treated control vines subjected to the same water-stress level. Considering that polyphenols, and flavonols in particular, have been associated with the plant response to water stress, the application of the biostimulant could mitigate the negative impacts through the concentration of powerful antioxidants in the leaf tissues.

Promising results were obtained in controlled conditions; further studies will be conducted in the field.

PNRR NODES Spoke 2, PoC PFS and GRINS PE0000018 - CUP D13C22002160001 are acknowledged.

102 - A beneficial role for thallium in the facultative hyperaccumulator Silene latifolia?

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The metallicolous populations of the facultative Tl hyperaccumulator Silene latifolia are extraordinarily tolerant and capable of accumulating up to 80,000 µg Tl g-1 in nature. A growth stimulatory effect of Tl was observed in this species and this study set out to determine possible mechanisms. Plants from non-metallicolous and metallicolous populations were subjected to hydroponics dosing experiments at 2.5 and 10µM Tl. Metal impact on stomatal and non-stomatal photosynthetic constraints, light energy conversion processes, and plant anatomy/ultrastructure was assessed over time. Photosynthetic rates improved in 10µM Tl-treated metallicolous plants by 20% compared to controls, partly due to increased stomatal conductance. The latter was mainly driven by Tl-induced anatomical changes, such as increased central cylinder area and stomatal density, likely to enhance water uptake/translocation and, consequently, leaf metal accumulation. The apparently Tl-favoured CO2 trafficking resulted in ameliorated maximal photosynthetic capacity. The first signs of photosynthetic declines appeared only at very high Tl leaf concentrations (15,000 µg Tl g-1), with limitations involving stomatal and biochemical factors whereas the photochemical reactions remained functional. The observed Tl-induced stimulatory response in growth and net photosynthetic rate in metallicolous plants shows that Tl appears to have a beneficial role in Silene latifolia, mainly linked to improved stomatal conductance.

104 - Mycotoxin contamination in maize can be predicted by analysing morphophysiological traits at different phenological stages

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Aflatoxins and fumonisins contamination in maize poses serious risks to food safety and crop value. In this study, we investigated 40 non-irrigated maize fields along a pedoclimatic gradient in NE Italy across the 2022 growing season to identify the phenological stage when morphophysiological traits can best predict final grain contamination. We sampled plant individual functional traits (*i.e.,* plant height, SLA, leaf dry matter content, leaf chlorophyll, carotenoid and flavonoid content, kernel dry weight, kernel C/N, kernel $\delta^{13}C$) at four phenological stages (*i.e.*, beginning of stem elongation, flowering, milk maturation, dent maturation). Moreover, we quantified kernel mycotoxins content (aflatoxins and fumonisins) at the harvest. We then created linear models for each phenological stage to relate mycotoxins with plant functional traits, soil composition and climate data (*i.e.,* total precipitation, mean temperature). Our goal was to assess which growth stage offers the highest predictive capability for final mycotoxin presence based on field conditions and plant status. Preliminary results suggest that trait–mycotoxin relations vary across development stages. However, a certain correlation with final contamination levels can already be observed at the beginning of stem elongation. These findings suggest that morphophysiological monitoring at selected phenological stages can support early risk assessment and management strategies to mitigate mycotoxin accumulation in maize.

105 - Responses of Alpine grassland plants to heat wave: analysis of selected physiological parameters.

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The Alps are facing more frequent summer heat waves, threatening alpine ecosystems and their biodiversity. These events impact primary alpine grasslands, key providers of ecosystem services. Recent studies show that combined drought and heat stress affects species and community-level responses, yet the role of stress timing and duration remains unclear. This study addresses this gap by examining heat wave effects on alpine grasslands at different growth stages, focusing on species from three functional types: sedges, grasses, and forbs.

Heat waves were simulated using shelters that excluded rainfall and increased temperature. Treatments varied in timing and duration: (1) early and prolonged (start to peak of season, ~2 months), (2) early (1 month after growth resumed), (3) late (1 month before peak), and (4) control (no stress). We assessed plant responses via morphological and physiological leaf traits, including root non-structural carbohydrates and pigment concentrations (chlorophylls, carotenoids, flavonoids).

We hypothesize that early stress may accelerate phenology and boost early growth, while prolonged or late exposure reduces productivity and induces early senescence. Forbs are expected to show the strongest functional responses, both morphologically and biochemically.
Our findings will improve understanding of alpine vegetation resilience under future climate extremes, guiding adaptive conservation strategies.

106 - Biochemical responses in durum wheat plants under drought stress conditions

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Durum wheat (*Triticum turgidum* subsp. *durum*) is one of the most widely cultivated and consumed crops worldwide. Drought stress severely compromises plant performance, leading to reduced photosynthetic capacity and metabolic imbalances that trigger the generation and accumulation of reactive oxygen species. Depending on factors such as cultivar, tissue type, and phenological stage, wheat plants activate specific physiological, cellular, and molecular responses aimed at minimizing drought stress, including hormonal changes and a general remodelling of both enzymatic and non-enzymatic antioxidant systems. Despite advances in common understanding, several mechanisms activated in response to drought stress are still not fully understood. In this study, biochemical analyses were conducted on leaf and root samples from two durum wheat cultivars (Svevo and Senatore Cappelli), subjected or not to drought stress.

Preliminary findings indicate a complex interplay between genotype and stress conditions, with differential responses observed at multiple levels.

Further investigations are needed to better elucidate the underlying mechanisms and to assess the potential implications for crop management and resilience strategies. In addition, it is also important to explore the interactions with rhizospheric microorganisms, such as arbuscular mycorrhizal fungi, which are believed to contribute to improved plant resilience under drought conditions as suggested by several studies.

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107 - miRNAs and salt stress: a study on Italian Rice Varieties

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MicroRNAs (miRNAs) are a class of small regulative RNAs that play key roles in modulating plant growth and adaptation to environmental cues. Elucidating the molecular mechanisms underlying plant's response to the environment is essential for crop improvement, given their critical socio-economic importance to ensure stable yields also under suboptimal conditions. Among the abiotic stressors, soil salinity has emerged as a major threat for crop productivity, in particular for rice (*Oryza sativa*), one of the world's most important staple crops. Intensive breeding programs have generated a broad range of rice varieties with varying degrees of salt tolerance, offering a unique source of genetic diversity. In this work, we use this genetic diversity to uncover the miRNA-based mechanisms behind rice response to salt. An initial screening identified salt-tolerant and sensitive Italian rice varieties, which were then used in a high-throughput sequencing experiment to detect the salt-regulated miRNAs. This allowed the identification of novel regulatory modules of the rice's response to salt stress, which include miR530, a salt induced miRNA, specific for the tolerant varieties. miR530 was already known for its role in regulating rice resistance to biotic stress and seed development, by targeting RMT1 and a PLUS3 protein downstream of the transcription factor PIL15. Our data show that the PIL15-miR530 pathway is also modulated during the rice response to salt stress, potentially influencing the rice's tolerance threshold. Overall, this work provides new insights into rice response to salinity, highlighting the importance of miR530 in fine-tuning the balance between stress resilience and growth.

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112 - To Oscillate or Not: Apoplast-ER Crosstalk Governs Stomatal Guard Cells Ca^{2+} Dynamics

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Stomatal opening is essential for plant survival, enabling CO_2 uptake for photosynthesis while minimizing water loss through transpiration. Stomata open in response to light and low CO_2 and close under drought, elevated CO_2, ozone, or pathogen attack. Stomatal closure involves both Ca^{2+}-dependent and -independent pathways, with cytosolic Ca^{2+} oscillations emerging as a hallmark of closed or partially open stomata and being absent in fully open ones. Although these oscillations were first described in the 1990s, their molecular mechanisms remain largely unresolved. Here, by performing live imaging in *Arabidopsis* and *Solanum lycopersicum* guard cells, we demonstrate that the occurrence of spontaneous Ca^{2+} oscillations is conserved in these two species. We further report, spontaneous Ca^{2+} transients in the endoplasmic reticulum (ER) lumen of guard cells. Moreover, the ER Ca^{2+} homeostasis is affected by light: blue light causes ER Ca^{2+} depletion, whereas red or green light, or darkness permit ER Ca^{2+} refilling, a process dependent on apoplastic Ca^{2+}. Our findings reveal that while apoplastic Ca^{2+} influx triggers cytosolic oscillations, their persistence requires a proper ER Ca^{2+} homeostasis.

Overall, this work underscores the interplay between extracellular and intracellular Ca^{2+} pools, for the generation and maintenance of cytosolic Ca^{2+} oscillations in guard cells, providing a foundation for further dissection of Ca^{2+} signaling in stomatal physiology.

120 - Next generation biostimulants in agromining: enhancing nickel phytoextraction in Odontarrhena decipiens (Nyár.) grown in serpentine soil

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Nearly 15% of global nickel (Ni) production is allocated to clean energy applications. Agromining—the use of hyperaccumulator plants to recover trace elements from contaminated soils—offers a sustainable solution for Ni extraction and recovery, but its scalability is often limited by the inherent low biomass and slow growth of hyperaccumulators. In this scenario, next generation biostimulants (microbial- and waste-derived products that can enhance plant nutrition and stress tolerance), present a promising yet underexplored innovation to overcome this limitation. This study investigated the influence of two biostimulants—Spirulina (the dried biomass of the cyanobacterium *Arthrospira platensis*) and wood distillate (WD, a wood pyrolysis by-product)— on the growth and shoot Ni accumulation in the hyperaccumulator species *Odontarrhena decipiens* (Nyár.) cultivated in a serpentine (Ni-rich) soil**.** Plants that were foliar sprayed with 0.5% WD (v/v) increased shoot Ni concentrations by 50% compared to water-treated controls, yielding 12,000 µg Ni per gram of dry weight. Moreover, soil fertigation with 1% Spirulina (v/v) enhanced total shoot Ni content by 250% (1,400 µg Ni plant^{-1}), primarily due to a remarkable >300% increase in biomass production compared to control plants. These results suggest that Spirulina and WD could synergistically maximize total Ni recovery from serpentine soils—leveraging both concentration-based and biomass-mediated uptake pathways for optimal agromining efficiency**.**

122 - From Waste to Wealth: Enhancing Plant Productivity with Digestate from the Organic Fraction of Municipal Solid Waste

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Soil degradation, characterized by a gradual reduction in organic matter, has long been recognized as a consequence of both natural processes and human activities such as deforestation and excessive land use. To mitigate these effects, the use of organic amendments is essential to increase soil organic matter and, thereby, enhancing soil fertility and crop yield. This project focuses on the valorisation of a by-product of the biogas industry—digestate from anaerobic fermentation—as an organic amendment to promote a more sustainable agriculture. This digestate originates from the organic fraction of municipal solid waste (OFMSW) and behaves as a soil improver, because its incorporation into the soil increases total organic carbon and water content with little impact on nutrient levels. The effectiveness of digestate as a soil amendment was initially evaluated using the model plant *Arabidopsis thaliana*. Concentrations ranging from 5% to 15% promoted plant growth under both in vitro and soil conditions. Further studies in Micro-Tom tomato plants assessed the impact of digestate on morphometric and productivity parameters—such as plant biomass and fruit production—along with biochemical indicators including chlorophyll, malondialdehyde, and polyphenols to gauge physiological responses. Overall, the results demonstrated that digestate application significantly increased plant biomass and fruit yield, highlighting its potential as a sustainable amendment in agricultural systems.

126 - Effect of drought-primed ecological memory on stress adaptation in tomato

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In the last years, the intensification of drought episodes in the Mediterranean area have seriously been challenging crop productivity and biodiversity. Though tomato physiological and transcriptional responses to single drought and rehydration events were studied, drought-primed ecological memory is poorly explored. This work investigates if repeated water deficit could prime the ability of tomato to endure drought. To this aim, tomato plants were exposed to a first water deprivation treatment (WS), followed by recovery. A second WS treatment was applied on the same plants (PRIMED) and on another group previously irrigated (UNPRIMED). Well-watered controls (CTR) were also included in the trial. Biometric, anatomical and physiological parameters were measured during both WS and recovery. Following the second WS input, PRIMED plants showed higher transpiration and assimilation than the UNPRIMED ones. Although PRIMED plants had a smaller total leaf area, such adjustment was compensated by a higher stomatal density compared to both UNPRIMED and CTR plants. Soluble carbohydrates and osmolytes did not differ significantly in leaf and root samples collected from all plant groups in both WW and WS conditions. Conversely, starch accumulation was opposite in the leaves of PRIMED and UNPRIMED plants based on the water regime. Integration of these data with whole transcriptome and DNA methylome analyses is ongoing to elucidate the establishment of stress memory signals further.

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127 - Priming grapevine adaptability to multiple stresses through somaclonal variability

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Grapevine faces increasing pressure from environmental and biotic stressors, calling for an improvement of breeding platforms. Somatic embryogenesis (SE; the initiation of embryos from somatic tissues) can generate new genetic variability, called somaclonal variation, which can be favored by adding priming agents *in vitro*, to regenerate genotypes with increased stress tolerance.
Here, we tested if vines *in vitro* regenerated through SE (*i.e.* somaclones) are prompter than the mother plant to endure abiotic cues. Water stress and recovery trials were conducted on somaclone lines of *Vitis vinifera* 'Nebbiolo' and of 110R rootstock, respectively regenerated in absence or presence of *in vitro* PEG selective pressure. Changes in eco-physiological responses (*e.g.* gas exchanges, relative water content, stem water potential) were monitored on somaclones and on the corresponding mother plants. Biometric and anatomical modifications were also checked and integrated with data of non-structural carbohydrates, defense secondary metabolites and hormones, to identify the most promising lines. In parallel, transcriptomic changes and genetic mutations potentially associated with tolerance traits were analyzed. The physiological performances of the selected somaclones were then confirmed in vineyard over two consecutive years. We demonstrated that the exploitation of somaclonal variability is an effective and ready-to-use genetic improvement strategy for implementing clonal selection in grapevine.

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

135 - Volatile Organic Compounds Drive Plant–Plant Communication in Drought Stressed Tomato

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Beyond conventional intraplant adaptive mechanisms, plants have evolved the ability to communicate with neighboring plants via volatile organic compounds (VOCs) in response to environmental stimuli. While numerous studies have demonstrated VOC-mediated signaling during biotic stress, evidence for similar communication under abiotic stress remains limited.

In this study we used a multidisciplinary approach to investigate the interplay among plant physiology, transcriptome and volatilome, with the aim to understand the mechanisms underlying plant VOC communication during drought stress. We investigated the responses of drought-stressed tomato plants (emitters) and the impact of their VOC emissions on neighboring, unstressed plants (receivers). The volatilome profiles showed that "emitters" released a higher number of VOC at higher concentration in comparison to the control condition. "Receiver" plants also differed in the VOC emissions in comparison to control plants for some drought stress markers. Gas exchange measurements on the "receivers" showed that the stomatal conductance was the main parameter to be affected by perception of emitters VOC. At transcriptome level, "receivers" activated numerous biological processes mainly related to cell wall, hormone metabolism as well as photosynthesis. In conclusion, VOCs emitted by stressed plants induced changes in gene expression, plant physiology as well as in VOC emission in not stressed companion plants.

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137 - Knocking out doubts: how to resolve the role of Lhcb2 in plant photoprotection

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The major light-harvesting complex of photosystem II (LHCII) is the most abundant pigment-protein complex on Earth, accounting for approximately 70% of the pigments bound to PSII. Composed of the Lhcb1-3 isoforms, LHCII is crucial for light harvesting regulation. It is also essential for photoprotective non-photochemical quenching (NPQ), which dissipates excess excitation energy as heat. It has been proposed that among the LHCII isoforms, Lhcb1 is fully competent to engage in NPQ, while the contributions of Lhcb2 and Lhcb3 appear negligible. Since Lhcb1 is the most abundant LHCII isoform, impaired NPQ in plants devoid of Lhcb1 may indirectly stem from an uneven distribution of the quenching sites within the pigment bed. By employing *Arabidopsis* lines missing Lhcb1, Lhcb2 or the entire trimeric LHCII, we investigated their role in catalyzing NPQ. In *koLhcb1,* total LHCII level decreased by 70% compared to the WT; however, the maximal NPQ was only slightly affected (-20%); moreover, the relative abundance of PSII supercomplexes remained consistent across WT, *koLhcb1* and *koLhcb2*. To simplify the relation between LHCII composition and amplitude of quenching, we examined the dependence of NPQ response by expressing various levels of Lhcb1 or Lhcb2 as the sole LHCII components. We found that maximal NPQ increased with increasing Lhcb1 levels, up to saturation at a LHCII content similar to WT. Contrary to previous reports, the expression of Lhcb2 as the sole LHCII isoform exhibited a dependence of maximal NPQ on LHCII content that closely matched that of lines expressing Lhcb1 only. Our results reveal that both Lhcb1 and Lhcb2 subunits contribute equally to the quenching response, implying that Lhcb2 is fully competent for NPQ activity.

140 - Exploring Plant-Plant Interactions in Intercropping Systems to Enhance Durum Wheat Growth and Drought Resilience

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Triticum durum is a widely cultivated cereal, in particular in Mediterranean Basin accounts for 60% of the global cultivation area. Despite its adaptability to various soils and environmental conditions, durum wheat is increasingly affected by drought events driven by climate change, particularly in Mediterranean regions. Water scarcity limits plant growth and physiology and also exacerbates the spread of fungal diseases.

Sustainable cultivation practices are needed to ensure food security while mitigating environmental impact. Intercropping (simultaneous cultivation of two species in the same field) represents sustainable practice displaying potential to optimize resource, reduce soil erosion, and suppress weeds.

In this scenario, the aim of this work is to evaluate the effect of intercropping on durum wheat cultivation under drought mainly focusing on mechanisms underlying plant-plant interactions. Having previously selected durum wheat genotypes with different drought tolerance degree, intercropping growing systems with clover has been established under both well-watered and drought conditions. The impact of intercropping was also tested by growing plant hydroponically in order to precisely modulate nitrogen input in the growth medium.

Preliminary results revealed that under drought, intercropping promoted the growth of wheat genotypes. To explore the plant-plant interaction mechanisms, chemical (ionome), biochemical (root exudome) and molecular analyses are in progress.

143 - Tissue-Specific Transcriptomics Reveals Developmental Mechanisms in Posidonia oceanica

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Posidonia oceanica is a key species in Mediterranean coastal ecosystems, offering habitat, stabilizing sediments, and storing carbon. However, its populations have declined by 34% over the past 50 years due to human impacts. Despite its ecological importance, little is known about the molecular basis of its development.

We performed transcriptomic analyses of roots, shoots, and seeds across four developmental stages, uncovering strong tissue-specific gene expression. Leaves showed active photosynthesis and stress response regulation. Roots were enriched in genes for carbohydrate metabolism and wax biosynthesis, aiding substrate adhesion. Seeds retained metabolic activity, with glycolytic enzymes suggesting germination readiness.

Co-expression network analysis identified tissue-specific gene modules: roots were enriched in lipid biosynthesis and cell wall remodeling; shoots in photosynthesis and development; seeds in metabolic maintenance and stress resilience.

Key regulatory hubs included ATP-citrate synthase and transcription factors MYB36, GATA12, and GRF1 in roots; PsbP-like protein 1 and WRKY22/72A in shoots; and Enolase, ZAT9, and NAC87 in seeds. These genes drive tissue-specific functions such as photosynthesis, iron homeostasis, and germination.

Our findings offer a foundational molecular framework for P. oceanica development and highlight targets for conservation and restoration.

145 - Deciphering Plant Interactions with Friends and Foes: the VOC Language

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Plants produce and emit many volatile organic compounds (VOCs). Volatile isoprenoids, making more than half of the plant VOCs: a) interact with biogeochemical atmospheric cycles leading to the formation of ozone, particles and other air pollutants; b) protect the photosynthetic apparatus and maintain leaf integrity under abiotic stresses, namely water stress, high temperatures and oxidative stress; c) change the hormone balance affecting plant growth and biomass above/belowground allocation; d) drive plant communication with other organisms, eliciting direct and indirect defenses against herbivores and pathogens; and e) allow plant-plant interactions arguably priming naïve receivers to readily respond to incoming stresses. The latter function would require VOC receptors in receiving plants. However, currently only few VOC receptors have been discovered. How the bulk of VOCs is sensed by plants remains unknown and must be unraveled, to be able to use VOC priming as a sustainable plant protection practice.

147 - Betalain degradation products (BDPs) as potential biostimulants able to improve salt and osmotic stress plant tolerance

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Abiotic stress negatively impacts plant growth and productivity, making it crucial to explore sustainable solutions able to improve plant resilience. Recently, we developed a novel biostimulant formulation derived from agri-food waste rich in betalain degradation products (BDPs). In this study, we investigated the effects of the formulation on growth and stress tolerance of *Arabidopsis thaliana* grown in agar plates under salt stress (SS) and osmotic stress (OS) conditions. The application of the formulation at different concentrations, significantly enhanced seedling growth and biomass. In particular, seedlings treated with 0.20 mg/mL showed a growth increase of +150 % and +116% under OS and SS, respectively. ROS level and antioxidant enzyme activity analyses revealed that the BDP-containing formulation modulated superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR) in both SS and OS. Moreover, the formulation affected proline metabolism by upregulating proline dehydrogenase (*PDH*), pyrroline-5-carboxylate dehydrogenase (*P5CDH*) and ornithine aminotransferase (*OAT*) genes. The overall results suggest that the formulation increased the abiotic stress tolerance of Arabidopsis seedlings by modulating oxidative stress responses and proline metabolism. Additional experiments are currently underway to explore the potential applications of this formulation in improving crop tolerance to adverse environmental conditions

150 - A moderate increase in sugar concentration may account for the osmotic adjustment observed in grapevine leaves under mild water deficit

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Water stress is the most recurrent abiotic threat in agriculture and is expected to increase due to climate change. Mediterranean viticultural regions are highly exposed to the drought risk. Understanding the mechanisms that enhance drought tolerance in *Vitis vinifera* is therefore crucial. Osmotic adjustment, which maintains cell turgor and gas exchange under water deficit, is a known strategy, but its regulation in grapevine remains unclear. Although various osmolytes have been identified in woody species, findings in grapevine are inconsistent. In this study, one-year-old Merlot grapevines were subjected to three distinct water regimes: (i) 100% of evapotranspiration (ET); (ii) 100% ET for 20 days, then 25% ET; (iii) 50% ET for 20 days, then 25% ET. Plants were monitored for several physiological traits to assess their acclimation. In the first fully expanded leaf leaves, we quantified key osmocompatible solutes contributing to osmotic potential. Results showed that osmotic adjustment was mainly associated with changes in sugar concentrations. Moreover, we found a coordinated response between leaf osmotic potential and petiole xylem anatomy: plants with more negative osmotic potential, showed narrower petiole xylem vessels. These findings highlight a complex but coherent adjustment to drought, where solute accumulation

153 - Polystyrene nanoplastics impact on crops: a new challenge for agricultural and food safety.

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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 [1]. Using molecular and biochemical analysis, the study aimed to investigate entrance and translocation of PSNPs in durum wheat, used as model crop, and their consequent effects on crop growth. Two lines were selected, Kronos and MRP3, a mutant with reduced phytic acid. PSNPs penetrated roots and translocated to shoots. 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 driving the altered carbohydrate metabolism, with upregulation of genes for methylglyoxal detoxification, a potentially toxic compound precursor of advanced glycation end products. Despite no visible detrimental phenotypic effects at this exposure duration, except for a lowered shoot length and biomass in the mutant, results highlight early markers of PSNPs-induced stress, indicating ongoing damage that may lead to yield losses, prolonging the exposure. Remarkably, MRP3 also exhibited photosynthetic impairment, exacerbating productivity threats. This study underscores the environmental and food safety implications of nanoplastics infiltrating the agri-food chain. On one hand, PSNPs in plants threaten crop productivity and agricultural sustainability by interfering with vital processes. On the other, they represent a risk to animals and humans due to the potential ingestion of contaminated food. These findings align with the One Health approach, highlighting the intrinsic interconnection between plant, animal, and human health within shared ecosystems.

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7 - Low external nitrate condition plays a positive role on symbiotic nitrogen fixation efficiency through the combined action of a NPF transporter subfamily

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A significant percentage of genes induced in Nitrogen-Fixing-Nodules encode for transport proteins and among these, at least nine members of the Nitrate/Peptide Transporter family (NPF) were identified (*LjNPF*-NI) [1]. In order to gain insights into the roles played by the *LjNPF*-NI genes, we traced their expression profile throughout the entire *L. japonicus* nodulation process, which was finely divided into six distinct stages based on the progressive increase in nodule size. The *LjNPF*-NI genes exhibit distinct profiles with peaks of expression at different stages of nodule development.

We previously reported the phenotypic characterization of *LjNPF8.6* and *LjNPF3.1,* whose knockout mutants display similar defective phenotypes in nodule functioning [2, 3], suggesting a potential positive role for these two members. We have now nearly completed the symbiotic phenotyping of mutants for all members of the *LjNPF*-NI sub-family, which now includes *Ljnpf5.2*, *Ljnpf5.3* and *Ljnpf3.4* as well as the double mutant *Ljnpf3.1/Ljnpf8.6*. The overall picture suggests a model in which *LjNPF*-NI members play combined roles in supporting the nodulation process under low-nitrate conditions. RNAseq analyses are in progress to compare the nodule transcriptomic profiles in wild type and *Ljnpf3.1/Ljnpf8.6* backgrounds.

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24 - Structural Barriers Against Xylella: Cell Wall Strategies in Olive Resistance

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Xylella fastidiosa (*Xf*) is a xylem-limited bacterial pathogen and the causal agent of Olive Quick Decline Syndrome (OQDS), a devastating disease severely affecting olive groves, particularly in the Mediterranean region. Although some cultivars exhibit resistance to *Xf*, the underlying molecular and structural mechanisms remain poorly understood. Recent studies have shown that phenolic extracts from olive mill waste exhibit antimicrobial activity against *Xf* and stimulate innate immunity in plants (Greco et al., 2024). Additionally, increasing evidence suggests a crucial role of the plant cell wall (CW) both as a physical barrier and as an active component of plant immunity (Lionetti et al., 2017; Coculo and Lionetti, 2022; Coculo et al., 2023; Del Corpo et al., 2024). In this study, we investigated CW structural traits in resistant (FS17) and susceptible (Arbequina) olive cultivars in response to *Xf* infection. Our findings indicate resistance involves active reinforcement of specific CW components. Overall, our results underscore the role of cultivar-specific CW dynamics in determining resistance outcomes and offer insights for breeding programs aimed at improving tolerance to *Xf*.

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45 - From agro-industrial by-products to immunomodulators for sustainable crop protection

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Olive mill waste (OMW) poses environmental concerns due to its high organic and phenolic content. While anaerobic digestion offers a sustainable route for biogas production, the environmental impact of the digestate remains unclear. In this study, two-phase olive pomace and its digestate were upcycled into bioactive immunomodulators for sustainable crop protection. Three extracts were obtained: a glycan-rich fraction containing immunostimulant oligosaccharides; a phenolic extract enriched in hydroxytyrosol, oleuropein, and related compounds; and a microbial protein extract from the digestate, containing MAMPs and DAMPs such as flagellin, EF-Tu, and Golven peptides. AThese extracts triggered immune responses in *Arabidopsis thaliana* and tomato, including MAPK phosphorylation, reactive oxygen species production, and upregulation of defense genes like *CYP81F2*, *WRKY53*, and *FRK1*. Pretreatment with these formulations enhanced Arabidopsis and tomato resistance to *Botrytis cinerea* and *Pseudomonas syringae*. The phenolic extract also showed broad-spectrum antimicrobial activity against key phytopathogens, including *Xylella fastidiosa*. These results represent the proof of concept for the Horizon Europe OLinWASTE project, which aims to develop a zero-emission system for converting olive mill waste into bio-based products such as immunostimulants, biopesticides, biofertilizers, bioplastics, and bioenergy.

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47 - Microplastics in soil alter crop pathogen susceptibility: evidence from Solanum lycopersicum

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We are living in a “plastic age,” where the widespread use of plastic polymers has led to the accumulation of microplastics (MPs) in terrestrial ecosystems. While increasing attention is being paid to MPs in the soil–plant system, their impact on plant disease resistance remains poorly understood. As agriculture shifts towards sustainability, it is crucial to assess whether MPs may compromise the innate immune responses of crops.

In this study, we examined the physiological effects of MPs on *Solanum lycopersicum* var. San Marzano Nano and their possible interactions with the plant response to pathogen infections. Tomato seedlings were grown in a growth chamber in soil amended with polyvinyl chloride (PVC) or polyethylene terephthalate (PET) MPs at 0.5, 1.0, and 1.5% w/w (40–50 μm size) for 14 days. The effects of MP-contaminated soil on tomato plants were comprehensively assessed through various growth and physiological analyses. Plant susceptibility was evaluated by infecting the plants with two pathogens: *Botrytis cinerea* and *Pseudomonas syringae* pv. tomato DC3000.

Although MPs did not significantly affect growth or photosynthetic efficiency, a dose-dependent decrease in chlorophyll and flavonoid content was observed. All MP concentrations altered the ionomic profile of the shoots. Notably, higher MPs levels enhanced *P. syringae* growth in infected leaves, while *B. cinerea* lesions increased only in PET-treated plants. To gain deeper insight into how MPs affect plant susceptibility to pathogens, a comparative transcriptomic analysis (RNA-Seq) was conducted on tomato leaves collected before and after infection. Although the transcriptomic analyses are still ongoing, our findings suggest that soil MPs can impair plant defense responses, increasing susceptibility to pathogens, with potential consequences for crop productivity and health.

58 - Investigating the Role of Endophytes in Enhancing Grapevine Resilience to Drought

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

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62 - Alternative N-forms cause differential response to the soil-borne disease Fusarium oxysporum f. sp. melongenae in eggplant (Solanum melongena L.)

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Mineral nutrition may strongly influence plant susceptibility to disease. Indeed, nitrogen (N) affects the plant-microbe interaction by driving the production of compounds that can modulate the disease development.

Comparative analysis of alternative N-forms (NO_3^- and NH_4^+) in response to the soil-borne pathogen *Fusarium oxysporum* f. sp. *melongenae* (*Fom*) were assessed in the tolerant eggplant line AM199. The NH_4^+-fed plants showed more evident disease symptoms compared to NO_3^--fed ones characterized by a reduced incidence. Root transcriptomic elucidated the defence mechanisms at the early stage (4 hours after inoculation; T1) and the cross-talk between plant responses to pathogen and the alternative N-forms at long-term (14 days after inoculation; T2).

At T1, we detected a rapid activation of genes involved in early defense, many of which mediate hypersensitive response and salicylic acid signaling, including the plant pathogen recognition complex and Pathogen-Associated Molecular Patterns. At T2, a boosted defense response in NO_3^--fed plants compared to NH_4^+-fed ones, characterized by both a higher expression of ROS and R genes, as well as the over-expression of genes involved in the cell wall development (*CSLG1*; *CSLH1*) and membrane sterols production (*SQE2*).

Our study provides new valuable insights into the molecular mechanisms induced by *Fom* infection under alternative N-forms supply, contributing to define the better fertilization practices for eggplant disease management.

65 - Hydrolysis of indole glucosinolates by PENETRATION2 modulates camalexin production and resistance to Botrytis cinerea in Arabidopsis thaliana.

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Plants produce a wide array of antimicrobial compounds to defend against pathogens and pests. In Brassicaceae, aliphatic and indole glucosinolates (AGS and IGS), derived from methionine and tryptophan, are converted, in response to infection or wounding, by myrosinases into isothiocyanates, nitriles, and other compounds with antimicrobial activity. In *Arabidopsis thaliana*, PENETRATION2 (PEN2) specifically hydrolyze IGS, contributing to resistance against different fungal pathogens. IGS share part of their biosynthetic pathway with the indole phytoalexin camalexin, but the regulation of their production and their relative contribution to defense are not fully elucidated. We found that *pen2* mutants show increased camalexin production and reduced symptoms when infected with the fungal pathogen *Botrytis cinerea*. This resistance is lost when *pen2* is crossed with the *pad3* mutant, defective in camalexin biosynthesis. Additionally, *pen2*-mediated resistance is also lost in a *cyp79f2* mutant background, defective in the biosynthesis of 4MI3G, a direct substrate of PEN2. These results suggest that the accumulation of non-hydrolyzed 4MI3G leads to enhanced camalexin production and increased resistance. These results highlight that feedback mechanisms fine-tune the accumulation of antimicrobial metabolites during pathogen infection.

73 - Zein-Based Nanoparticles for the Delivery of Bioinsecticides in Sustainable Crop Pest Control

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To limit the use of pesticides and to develop strategies for controlling plant pests and pathogens, researchers have identified some bioactive molecules by exploiting insect-natural antagonist interactions as source of bioinsecticide molecules. These include a dsRNA that silences an immune gene in the chewing insect *Spodoptera littoralis* and peptides derived from parasitoids with neurotoxic effects on caterpillars.

Due to their high sensitivity to environmental degradation, these bioactive molecules must be protected by sustainable and eco-compatible formulations for effective field application. Such formulations should promote bioactive molecules' accumulation into plant tissues and guarantee their delivery into the target insect without compromising their bioinsecticide activity.
Here, we present the characterization of zein-based nanoparticles (NPs) using tomato plants as a model crop.

By using a multidisciplinary approach that integrates microscopy techniques, physiological and molecular analysis, we demonstrate that:

- NPs rapidly penetrate tomato leaves, localize within the apoplast, and do not alter plant physiological parameters up to one week post-delivery by foliar spraying;
- NPs require approximately four hours to transit through the larval gut;
- NPs remain stable under acidic conditions, such as the one of leaf apoplast, but become rapidly unstable under alkaline pHs resembling those of *S. littoralis* larval midgut;
- NPs do not impact the viability of model organisms such as earthworms, zebrafish or human skin and lung epidermal cell lines, thus being fully eco-compatible

91 - Role of the Salt Tolerance-Related Protein (STRP) in the responses of Arabidopsis thaliana to infection by Pseudomonas syringae pv. tomato DC3000

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The Salt Tolerance-Related Protein (STRP) is a small (16 kDa), intrinsically disordered protein of *Arabidopsis thaliana,* which has been shown to play a protective role against cold and salt stress. However, its role in biotic stress responses remained unexplored. Hence, here we investigated its role in the response to infection by the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000), a model organism for studying plant-pathogen interactions.

Upon *Pst* DC3000 infection, STRP rapidly accumulates in the cytosol and nucleus. RT-qPCR and treatment with the proteasome inhibitor MG132 revealed that protein accumulation was not due to increased transcription, but rather to reduced degradation via the 26S proteasome. Reverse genetics experiments using the *strp* knockout mutant and *STRP*-overexpressing (*STRP* OE) lines showed that STRP limits disease symptoms, since necrotic lesions were larger in *strp* mutants and reduced in *STRP* OE plants with respect to wild-type plants. STRP also modulates typical Pattern-Triggered Immunity (PTI) responses, such as stomatal closure and callose deposition, which were impaired in *strp* mutants but enhanced in *STRP* OE plants. Consequently, *strp* mutants exhibited higher endophytic colonization.

Overall, the findings demonstrate a positive correlation between STRP levels and the activation of plant defense responses to *Pst* DC3000, suggesting a novel role for STRP in biotic stress tolerance mechanisms in *Arabidopsis thaliana*.

124 - Halophilic Microalgae from Trapani Salt Ponds: Bioactive Potential and Biostimulant Applications in Sustainable Agriculture

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Keywords: plant growth-promoting, halophilic microalgae, biological activity, biostimulant.

In this study, five microalgae strains were initially screened for plant growth-promoting (PGP) traits, including salt tolerance, indole-3-acetic acid (IAA) production, and phosphate solubilization. Based on their promising in vitro performance, three strains—*Dactylococcopsis salina*, *Dunaliella* sp. (both isolated from the salt ponds of Trapani[1]), and *Chlorella* sp. (from the Sicilian coast)—were selected for further in vivo experiments. Bioassays conducted on tomato seedlings (*Solanum lycopersicum* cv. *Regina Ostuni*) demonstrated that all three strains significantly enhanced key growth parameters, such as shoot biomass, primary root length, and hypocotyl elongation. These findings highlight the potential of halophilic and halotolerant microalgae as sustainable biostimulants for improving crop performance under saline and arid conditions.

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129 - Unraveling Tolerance and Susceptibility in Olive Cultivars: A Multi-Level Analysis of Xylella fastidiosa Infection

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This study investigates the interaction between the olive tree (*Olea europaea*) and the bacterium *Xylella fastidiosa*, the pathogen responsible for "Olive Quick Decline Syndrome" (OQDS), by comparing the responses of two cultivars: the tolerant Leccino and the susceptible Cellina di Nardò. Our objective was to elucidate the physiological, biochemical, and molecular mechanisms that underpin their contrasting reactions to infection. Preliminary biochemical analyses reveal that infection significantly alters critical metabolic processes in both cultivars. Infected plants show marked reductions in photosynthetic pigments (including chlorophyll a and carotenoids) as well as a decreased polyphenol content, indicating interferences in both primary and secondary metabolic pathways. Notably, in Leccino flavonoid levels decline sharply, whereas in Cellina di Nardò they remain relatively stable. Moreover, lipid peroxidation assays demonstrate lower malondialdehyde (MDA) concentrations in infected Leccino, suggesting the activation of a more efficient antioxidant defense. High-quality transcriptomic data were generated via RNA sequencing (150 bp paired-end, 16 libraries, n = 4 per group), with each library yielding between 26 and 40 million reads and Phred scores nearing 40. This analysis revealed consistent and reproducible differences between infected and healthy samples, as well as between the two cultivars.

Future research will focus on dissecting gene expression linked to anatomical modifications and biotic stress responses and on assessing key antioxidant enzymes to provide a comprehensive understanding of the mechanisms governing tolerance and susceptibility to *Xylella fastidiosa* infection.

132 - Assessment of arbuscular mycorrhizal symbiosis in the tolerance of different durum wheat genotypes subjected to environmental stress conditions

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In the Mediterranean and semi-arid regions, durum wheat (*Triticum turgidum* subsp. *durum*) is increasingly exposed to climate-related stresses such as drought and phosphorous (P) deficiency. Arbuscular mycorrhizal fungi (AMF) represent a potential strategy to enhance plant stress tolerance, though effectiveness depends on stress severity and host genotype interaction. To investigate this beneficial interaction, two experiments were performed in greenhouse. The first assessed the AMF-mediated response of eight durum wheat genotypes inoculated with *Rhizophagus irregularis* under three water levels (not-stressed, water-stressed and waterlogged). Biometric and ecophysiological parameters were monitored, and leaf and root samples were collected for RNA-seq and biochemical analyses, and root colonization assessment. Preliminary results showed that four genotypes were influenced by both the stress conditions and presence of AMF, showing genotype-dependent responses. In a parallel experiment, three Tunisian landraces and three commercial cultivars were inoculated with *Funneliformis mosseae* and grown under high or low P availability. RNA-seq of roots showed distinct profiles, depending on P condition and AMF presence. Both studies showed an effect of AMF, depending on host genotype in plant stress response, contributing to identify resilient lines for sustainable agriculture. Ongoing analyses will explore regulatory networks driving symbiosis and stress response.

133 - CELLOX1-mediated modulation of DAMPs signaling fine-tunes root immunity and shapes interactions with beneficial and pathogenic fungi

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Plants constantly interact with fungi, which exert beneficial, neutral, or detrimental effects on their survival. A critical checkpoint in this process is the modulation of plant immune responses. Damage to plant cell walls (CW) triggers the release of damage-associated molecular patterns (DAMPs), which are recognized by the plant and activate immunity. While the role of CW DAMPs towards pathogens is widely characterized, their role towards beneficial fungi remains understudied. In *Arabidopsis thaliana*, the berberine-bridge enzyme-like CELLOX1 oxidizes the CW-derived DAMPs cellodextrins and mixed-linked glucans, impairing their immunogenicity and thereby regulating immunity. This may enhance resistance to pathogenic fungi by converting these DAMPs into a less digestible carbon source as well as by promoting H_2O_2 production. Here, we show that CELLOX1 regulates root immunity and influences the interaction with the beneficial fungus *Serendipita indica*. Loss of *CELLOX1* enhances both intra and extra-radical fungal colonization, leading to stronger growth-promotion and protection against the pathogen *Bipolaris sorokiniana*. This is accompanied by increased immune responses and *S. indica*-induced root cell death. Conversely, *CELLOX1* overexpression reduces *S. indica* colonization, diminishes beneficial effects and weakens immunity. Our results uncover a novel role for CELLOX1 in fine-tuning root immunity and shaping the outcome of the interaction with beneficial and pathogenic fungi.

139 - Dendrosurgery as a Target Treatment for Esca Disease in Barbera: A Case Study

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Grapevine (*Vitis vinifera* L.) is affected by several fungal diseases, including esca, a severe trunk vascular disease that leads to leaf stripes, wood decay, disrupting vine physiology and, in severe cases, plant death.

Esca disease causes economic losses due to reduced yields and the costs associated with replacing diseased or dead grapevines. Therefore, the removal of infected wood is crucial to limiting the spread of the disease. Recently, alongside the use of preventive strategies, such as selected tolerant rootstocks, a new approach has been introduced: the dendrosurgery (DDC). This practice involves the mechanical removal of necrotic wood. Uncertainties remain regarding the short and long-term effects of the DDC treatment on the productivity, symptom occurring and physiological performance of treated grapevines.

This study focused on *Vitis vinifera* cv. Barbera vines (Azienda Braida, Rocchetta Tanaro, AT, Italy) reporting esca symptoms, which were treated 3–4 years before using dendrosurgery (DDC). These DDC-treated vines showed no esca disease symptoms since the treatment. Additional symptomatic vines were treated with DDC in 2025. Leaf gas exchange, stem water potential, and photosystem damage were assessed in these plants at the beginning of the growing season and compared with asymptomatic vines. Preliminary analyses did not reveal significant differences in the measured parameters, suggesting that DDC may be an effective defense strategy against esca disease. Further ecophysiological measurements, anatomical and productivity-related analysis, will be carried out during the growing season.

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142 - Role of oligogalacturonides (OGs) and their oxidation by OGOX enzymes in systemic plant defence responses

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Cell wall damage caused by wounding or pathogens releases Damage-Associated Molecular Patterns (DAMPs), including oligogalacturonides (OGs), which activate local immune responses via pattern recognition receptors (PRRs). OGs accumulate through the combined action of microbial polygalacturonases (PGs) and plant PG-inhibiting proteins (PGIPs). The activity of OGs is tightly regulated: OG oxidases (OGOXs), a class of Berberine Bridge Enzyme-like proteins, inactivate them to prevent excessive immune responses that could impair growth or trigger cell death. While OG-triggered local defenses are well described, their ability to induce systemic signals remains unclear. Long-distance signaling from local stress may involve calcium waves, ROS, hydraulic and electric signals, and is often associated with JA/JA-Ile accumulation and expression of wound-responsive genes. This study investigates the involvement of OGs in systemic defense by analyzing distal responses such as stomatal closure, systemic gene expression activation, and resistance to pathogen infection. Furthermore, we explore the role of OG oxidation by OGOXs in modulating these systemic effects, potentially acting as a negative feedback mechanism to fine-tune plant immunity without compromising growth.

149 - Oligogalacturonides: a pleiotropic signal involved in immunity and development through their interaction in the cell wall with diverse proteins and peptides

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Most biological events are regulated by signal molecules that first reach optimal concentrations, then bind specific receptors, and finally are removed after signaling. Plant hormones are highly pleiotropic, as a result of complex interactions between receptors, concentration, cross-talk, tissue specificity, and temporal regulation. Pleiotropy is also featured by plant-derived signals other than hormones such as the oligogalacturonides (OGs), prototypical damage-associated molecular patterns (DAMPs) that induce a PTI-like response in a size-dependent manner. OGs released in the apoplast by microbial polygalacturonases (PGs) interact with oligogalacturonide oxidases (OGOXs), PG inhibitors (PGIPs), WAK and WAK-like receptors, and rapid alkalinization factors (RALFs). Developmental processes are affected by the ability of OGs to antagonize auxin action. During infection, plants rely on a unique biological strategy that drives two proteins of organisms from different kingdoms (microbial PG and plant PGIP) to form a dimeric enzyme that favors the formation of DAMP-active OGs. Whether a similar heterodimer is formed in plants in the absence of microbes in specific developmental contexts is not yet known. Plants have evolved redundant OGOXs that dampen the activity of OGs when no longer necessary. The complexity of the biology of OGs is being studied in our laboratories by investigating their allostasis and homeostasis in the cell wall.

151 - Characterization of endophytic microbiota in seeds and tissues of the seagrass Posidonia oceanica

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Endophytic microorganisms, including bacteria, fungi, and actinomycetes, commonly colonize plant tissues, establishing mutualistic relationships that provide beneficial effects to the host plant. However, such interactions are still poorly understood in marine plants. The Mediterranean seagrass *Posidonia oceanica* hosts a complex endophytic community in adult individuals, but the composition of the microbiota associated with its main organs - leaves, roots, and seeds - during early developmental stages remains largely unknown.

This study investigated the microbiota of *P. oceanica* through metagenomic analysis of the three plant tissues and isolation of the culturable fraction obtained from sterilized and homogenized seeds. Molecular identification was carried out via sequencing of the 16S rDNA gene (for bacteria) and ITS/LSU regions (for fungi), while metagenomic data from the three tissues are currently under analysis. Regarding the culture-dependent approach, 44 strains were isolated on saline media and selected based on their morphology. Their identification revealed 18 halophilic or salt-tolerant bacterial species distributed among 8 genera, as well as two fungal species belonging to the marine genera *Paecilomyces* and *Halophytophthora*.

Some of these species are known for their beneficial effects on plants and could be exploited in *P. oceanica* conservation programs, particularly for seed preservation, seedling development, and pathogen protection.

06 NEW FRONTIERS IN GREEN BIOTECHNOLOGIES

9 - Impact of Constitutive Zeaxanthin Accumulation on Non-Photochemical Quenching and Photosynthetic Efficiency in non-model species Chlorella vulgaris.

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Photosynthetic organisms rely on efficient light harvesting and photoprotection mechanisms to optimize energy utilization and protect against oxidative damage. Carotenoids play crucial roles in these processes by broadening the light absorption range, acting as antioxidants, and participating in non-photochemical quenching (NPQ) to dissipate excess energy. While the roles of carotenoids have been extensively studied in model species, their functions in non-model microalgae remain less understood. In this study, we isolated by UV mutagenesis a mutant strain of the microalga *Chlorella vulgaris*, named *fm53*, constitutively accumulating zeaxanthin, lacking other β-β xanthophylls. Whole Genome Sequencing revealed a single point mutation in the 5' UTR of the zeaxanthin epoxidase gene, resulting in the destabilization of RNA transcription and the absence of the protein. Besides an altered carotenoid composition, the *fm53* showed a rearranged photosynthetic complex organization with a higher dissociation of the PSII supercomplex and a reduction of its maximum efficiency. NPQ and its fast component qE were strongly reduced in the mutant because of the constitutive quenching due to zeaxanthin. Despite these differences, biomass and carotenoid yields were comparable between the Wt and *fm53* under various growth conditions, suggesting that this pigment accumulation can compensate for the absence of other xanthophylls. This study provides new insights into the role of different xanthophylls in light harvesting and photoprotection mechanisms and highlights the significance of zeaxanthin in NPQ induction and photosynthetic efficiency in non-model microalgae.

15 - Cell suspension cultures of Coffea arabica L. as a source of extracellular vesicles

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Cell suspension cultures (CSCs) from *Coffea arabica* L. have recently been established in our laboratory (Di Bonaventura et al. 2024). In addition to being a valuable source of secondary metabolites, we found that these CSCs also release extracellular vesicles (EVs) into the culture medium. Following filtration of the CSCs, EVs were isolated by differential centrifugation, first at 100,000 × *g* for 36 minutes (fraction 100k *g*), and then at 125,000 × *g* for 6 hours (fraction 125k *g*). The two EV fractions were very similar in terms of size, shape, and ζ-potential. SDS-PAGE and Western blot analyses revealed the presence of H^+-ATPase and calnexin 1/2 in both fractions, serving as markers for the plasma membrane and endoplasmic reticulum, respectively. Proteomic analysis identified nearly 500 proteins in the 100k *g* fraction and approximately 300 in the 125k *g* fraction. Of these, 232 proteins were shared with both fractions, while 267 and 71 proteins were unique to the 100k *g* and 125k *g* fractions, respectively. STRING analysis indicated that the 232 shared proteins were predominantly enriched in the extracellular region. The two fractions were enriched in proteins associated with the cell periphery and plasma membrane (100k *g*), or localized to the extracellular region (125k *g*), respectively. These findings demonstrate that CSCs from *C. arabica* represent a valuable and scalable platform for the production of EVs, which may have potential biotechnological applications.

16 - PHASEOLIN, A NEW ECO-FRIENDLY PROTEIN-BASED BIOPLASTIC

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Keywords: biopolymer, disulfide bridges, phaseolin

Petroleum-based materials represent today one of the major sources of pollution for the environment. Therefore, the scientific community is constantly looking for strategies to produce new eco-friendly bioplastics that can replace petroleum-based materials, and this present work proposes a strategy that could partially replace them.

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

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

25 - Flavodiiron protein activity outcompetes cyclic electron transport when expressed in angiosperms

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The rate of photosynthesis is one of the main factors impacting yield in crop plants. In conditions of excess illumination, alternative electron transport pathways in the thylakoid membranes protect the photosynthetic apparatus against damage from eventual over-reduction. Two main pathways downstream of photosystem I (PSI) enable alternative electron flow, mitigating PSI photodamage: cyclic electron transport (CET) and pseudo-cyclic electron transport (PCET). Flavodiiron proteins (FLV) are crucial enzymes in PCET, found in all photosynthetic organisms but lost during the evolution of angiosperms. The absence of FLV coding sequences in angiosperm genomes raises intriguing questions about their role in photosynthetic organisms. Previous studies utilizing heterologous expression have already demonstrated that FLV can function in angiosperms. In this study we expressed FLV proteins from *Physcomitrium patens* in *Nicotiana tabacum* and *Solanum lycopersicum* and analyzed their impact on PSI activity under fluctuating light conditions. Transgenic lines exhibited significantly increased PCET rates, with FLV-dependent electron transport competing for electrons with CET, particularly under sudden increases in light intensity. These findings suggest not only that FLV are active in a heterologous system, but also that they can act as an additional electron sink and play a critical role in protecting the PSI of crops from over-reduction under fluctuating light conditions.

30 - LED spectral recipes to counteract the temperature stress in tomato seedlings

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Seedling quality is fundamental in horticulture as it might affect the growth and yield after transplantation. Light quality and quantity influence the plant development and physiology. In the last decade, LED lighting has provided great support to horticulture and research. This project aims to investigate how different LED treatments affect the resilience to temperature stress in tomato plants.

The growth of tomato seedlings of two Apulian varieties (Regina and Mola) was investigated under two specific narrow LEDs (red and blue) and a full-spectrum one (white). Two light intensities (100 and 200 μmol m^{-2} s^{-1}) and three temperatures (20, 25, and 30°C) were considered. Morphometrical and biochemical analysis, such as growth parameters and markers of oxidative stress, were carried out at the early growth stages. The obtained results highlight that blue light consistently induced the highest oxidative stress and significantly inhibited both shoot and root growth. In contrast, red and white light promoted a more balanced plant development, with lower stress markers and better growth performance. These findings point out that the selection of the best LED spectral recipe is an effector that can be leveraged to sustain tomato seedling growth, maximizing thermal stress resilience.

Keywords: Tomato; Light emitting diodes; Temperature stress; Oxidative stress

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44 - Putrescine as biostimulant agent in Cucumis sativus

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The ever-increasing global demand for food, combined with the rise of abiotic stresses due to ongoing climate change, underlines the urgent need for sustainable agricultural practices aimed at enhancing crop productivity. Plant biostimulants (BSs) represent promising eco-friendly alternatives to synthetic fertilizers. Among them, polyamines (PAs) such as putrescine (Put) have gained attention. PAs are biogenic, ubiquitous amines involved in growth regulation and defence responses. In this study, we investigated the effect of foliar application of exogenous Put on adult cucumber plants (*Cucumis sativus*) grown under both optimal and salt stress conditions. Phenotypic traits and biochemical stress markers were analysed to assess plant responses. Overall, Put-treated plants exhibited improved growth compared to controls under salt-stress conditions, particularly showing enhanced root development. Biochemical markers of plant fitness, including malondialdehyde content, antioxidant activity (FRAP assay), and photosynthetic pigment levels, indicated a better acclimation of Put-treated plants to salt stress. These results suggest that foliar application of exogenous Put can enhance plant resilience and performance by influencing phenotypic plasticity and modulating biochemical pathways, thereby offering a promising strategy to improve crop yield and health under adverse environmental conditions.

63 - Production of human alpha-mannosidase in transgenic tobacco plants: a semi-industrial approach

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Recombinant protein production is a key focus in modern biopharmaceuticals, with host selection driven by economic considerations and protein complexity. Here, we used a human lysosomal enzyme (α-mannosidase) as a model protein to evaluate an innovative approach for its production in engineered tobacco plants. Specifically, indoor hydroponics was chosen as cultivation system because it can be easily scaled up in an industrial setup. At 35 cm height, plants underwent cutting and regeneration (C&R) to boost biomass while preserving α-mannosidase synthesis. In pilot-scale trials, transgenic tobacco at 250 plants/m^2 produced 77 kg/m^2/year of biomass, which increased to 143 kg/m^2/year with C&R. In an industrial setup, 250 plants/m^2 yielded 147 kg/m^2/year during regeneration step, with potential for further increases via CO_2 enrichment up to 213 kg/m^2/year at 10000 ppm (CO_2). We detected α-mannosidase and its proteolytic fragments in leaf tissue, with yields of 1.4 µg/g FW for full-length protein and 18.8, 9.2, and 1.8 µg/g FW for 60, 32, and 18 kDa fragments, respectively, in the full growth cycle and showed the presence of proteolytic fragments in regenerated plants. The data indicates that α-mannosidase is regularly expressed in hydroponically grown transgenic tobacco with the accumulation of 0.2 g/m^2/year. Furthermore, implementation of C&R together with CO_2 enrichment can significantly increase biomass yield in indoor cultivation system.

71 - Phenylpropanoid Pathway Reconstruction in Yeast: Elucidating Novel Reactions for the Production of Hydroxycinnamic Acid Conjugates

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Specialised metabolites are a rich source of bioactive compounds which play critical roles in plant adaptation. Among these, hydroxycinnamic acids (HCAs) conjugates represent a relevant class of compounds produced during plant stress responses. However, most biochemical steps of their production remain unknown. This study aims to elucidate unexplored reactions in phenylpropanoid biosynthesis to enable their production in engineered yeast. *S. cerevisiae* CEN.PK2-1D was modified to recombinantly express naringenin chalcone synthase (CHS) and coumaroyl-CoA ligase of *S. lycopersicum*. Using feeding experiments, we investigated the catalytic potential of CHS in forming C-N bonds by supplementing cultures with p-coumaric acid and putrescine. In addition, the functional role of an uncharacterised protein showing a conserved HXXXD acetyltransferase motif was also assessed. Particularly, we tested its ability to catalyse the esterification of cyanidin-3-O-rutinoside with HCAs, observing notable colour changes in liquid cultures expressing HXXXD gene. These results indicate that HXXXD-dependent molecular decoration may enhance anthocyanin stability under variable pH conditions. Future structural and analytical studies will aim to validate the identities of these newly formed compounds. This work contributes to a deeper understanding of phenylpropanoid biosynthesis in heterologous hosts and lays the foundation for developing yeast-based platforms to produce high-value plant metabolites.

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74 - Biotechnological Recycling of Exhausted Cell Culture Media Using Microalgae – BioReMediA

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Every laboratory focusing on biomedicine worldwide makes usage of cell cultures. Biological and biomedical investigations are typically so important that the gargantuan amounts of liquid waste produced by research labs all over the world are practically unnoticed. Our idea sparks from such awareness, triggered by reflections about the economic and environmental impact caused by the daily disposal of large amounts of exhausted cell culture media (ECCM).

BioReMediA is a medium-TRL project for recycling ECCM exploiting microalgae. We have explored whether the high versatility of microalgae in detoxification and bioremediation of contaminated effluents could be applied to ECCM treatment. From the very first experiments, we have found that microalgae can grow efficiently on formulations containing up to 80% ECCM, and that several ECCM ingredients effectively boost microalgal growth, achieving 3- to 9-times higher biomass than control experiments using standard microalgae growth medium. We found out that in ECCM microalgae also accumulate higher amounts of pigments, lipids and carbohydrates. Thus, this project offers extensive opportunities of transforming ECCM into value-added products, including biofuels, fertilizers and fine chemicals.

Not only: we found out that microalgae are able to remove efficiently nitrogen, phosphorus and to reduce the chemical oxygen demand in the ECCM.

BioReMediA provides strategies to convert the straight usage-disposal path of cell culture media into an unprecedented circular approach to handle these bio-based reagents, offering them a “second life” as supplements to enhance microalgal biomass growth.

76 - Harnessing Chlorella vulgaris in a two-stage bioprocess for the sustainable valorisation of dairy waste: integrating biomass recovery and ethanol production

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The urgent need to reduce the environmental impact of agro-industrial waste is drawing increasingly focused attention to the sustainable valorisation of dairy processing by-products. Dairy wastewater generated from cheese manufacturing poses significant disposal challenges due to its high its high lactose and proteins content as well as its high biochemical and chemical oxygen demand; hence, the identification of sustainable and circular valorisation strategies is essential. Here we report a two-stage bioprocess involving Chlorella vulgaris and ethanologenic bacteria for the integrated recovery of microalgal biomass and ethanol from dairy waste. The proof-of-concept aimed to introduce microalgae at two stages in the waste reuse process, before the bacterial fermentation of the raw dairy waste and after fermentation. In the first stage, C. vulgaris was cultivated in raw and diluted waste dairy under controlled conditions, achieving higher biomass yields than those obtained in photoautotrophic growth with respect to the microalgal control medium. In the second stage, microalgal cultivation was performed in diluted fermentation effluent, showing comparable or increased growth with respect to its growth in microalgal control medium. Ethanol production was also evaluated before and after microalgal cultivation, revealing significant yields in all tested conditions. The results obtained with this approach confirmed that microalgae can be effectively integrated into a circular economy context at two stages of the process, offering a promising model that combines waste recovery, biofuel production, and biomass generation within a single, cost-efficient platform.

81 - From Dark to Light: Structural and Functional Adaptations of Photosystems in Galdieria phlegrea

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Galdieria phlegrea is a unicellular extremophilic red algae able to autotrophic, heterotrophic, and mixotrophic growth. This study investigates the physiological and structural differences in the photosynthetic apparatus of *G. phlegrea* during the transition from heterotrophy to autotrophy. Heterotrophically grown cultures were exposed to light without organic carbon sources and monitored for six days. The maximum quantum efficiency of PSII in the dark (Fv/Fm), and the effective quantum yield of PSII in the light (Y(II)) were significantly higher under autotrophic than in heterotrophic conditions, as was the chlorophyll content. In heterotrophic conditions, despite PSI abundance was lower compared to its level under autotrophic conditions, transient absorption (Pm') and Y(I) measurements, and 77K fluorescence spectroscopy and confocal microscopy images showed that PSI complexes remained fully active.

These results highlight a dynamic reorganization of the photosynthetic apparatus, enabling *G. phlegrea* to rapidly respond to environmental changes and optimize energy metabolism under light driven conditions.

88 - Isolation and characterization of soil bacteria as potential biocontrol agents against Fusarium cerealis and Fusarium sambucinum, two phytopathogenic fungi

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Bacteria are the major components of soil environments, possessing many of the enzymes needed to degrade organic matter and, in particular, cell walls. Chitinolytic and cellulolytic soil bacteria producing cellulases and chitinases, enzymes able to break down plant and fungal cell walls, could be considered as potential biocontrol agents. Within the ONFOODS project, 150 bacterial strains isolated from soils collected in the protected area of Pollino and Sila National Parks, in the Mediterranean area, a well-known hotspot of biodiversity, were screened for their ability to produce and secrete cellulolytic and chitinolytic enzymes. The screening criteria included the formation of clear zones on carboxy methyl cellulose and Avicel® PH-101 microcrystalline cellulose agar plates, as well as the spectrophotometric quantification of different enzymes production including N-acetyl glucosaminidase and β-glucosidase. Furthermore, the antagonistic activity of bacterial strains was evaluated to assess their potential application as biocontrol agents against two phytopathogenic fungi belonging to the *Fusarium* genus, *F. sambucinum* and *F. cerealis*. Isolation and characterization of a few members of these microbial communities, especially in biodiversity-rich environments such as the Mediterranean basin, could offer new strategies for biocontrol and sustainable crop management and explore the potential biotechnological applications of these bacteria.

96 - Investigation of organic dye acting as artificial antenna in green algae and cyanobateria

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Microalgae-based biotechnology can play a pivotal role in future low-carbon economy through Carbon Capture and Utilization (CCU) technologies. Recently, a new approach emerged using synthetic organic dyes as artificial antennae to boost photosynthetic efficiency in diatoms grown in close photobioreactor systems, overcoming current limitations of autotrophic growth. Our work aims to translate and validate this strategy in green algae and cyanobacteria, two groups of high biotechnological relevance. Among others, Cyanine 5 (Cy5) was selected for model green algae (Chlorella sorokiniana CCAP 221/8k), while Fluorescein was chosen for model cyanobacteria (Synechocystis sp. PCC6803) wild type and antenna-mutants strains (namely PAL, OLIVE, PAL, ΔCK and ΔCB), based on toxicity, biocompatibility and spectral properties. The effectiveness of the antenna insertion to broaden the absorption spectra of the photosynthetic systems with consequences on the photosynthetic activity was tested by means of advanced optical techniques: excitation spectroscopy with a fluorometer and time-resolved photoluminescence spectroscopy through a streak camera have been done in bulk. To assess the single-cell localization of the artificial antenna, characterisation was performed with a custom-made patent-based hyperspectral microscope. Promising results were obtained in particular in green algae, showing energy transfer between Cy5 and the chlorophyll consistent with enhanced photosynthetic performance.

101 - Heterologous production of polycyclopropanated fatty acids in Chlamydomonas reinhardtii

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The global rise in CO_2 emissions has significantly contributed to climate change, urging the scientific community to pursue sustainable energy alternatives. Among these, biofuels have attracted growing interest, but the energy density limits their use in aviation and aerospace sectors, which still depend on petroleum-derived fuels rich in cyclic and branched alkanes. This study investigates the potential of engineering *C. reinhardtii* to produce fuelimycins, novel, energy-dense polycyclopropanated fatty acids (POP-FAs) with potential as sustainable jet fuels. Fuelimycins - a C18-C20 chain with 6–7 cyclopropane rings - biosynthesis involves four genes (POP1–POP4) from *S. albireticuli*, encoding an iterative polyketide synthase responsible for fatty acid elongation and cyclopropane ring formation.

We successfully integrated the POP gene cluster into the nuclear genome of *C. reinhardtii* UVM4 and imported the products into the chloroplast. Fluorescent reporters were fused to the transgenes to monitor expression *in vivo*, and long-term stability was assessed under selective pressure. HPLC-QMS analysis revealed ions corresponding to unsaturated POP-FAs, although the precise structure was not fully characterized. Notably, we identified putative compounds such as C16:CP5, CP12:CP3, and C12:CP4, which are shorter and have fewer cyclopropane rings.

These findings indicate that while the biosynthetic pathway is functional, further optimization is necessary to enhance fuelimycins production. Additionally, the results underscore the potential of *C. reinhardtii* as a heterologous expression platform for producing POP molecules.

108 - Assessing validation methods for RNA guides in CRISPR-Cas9 genome editing of durum wheat

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CRISPR-Cas9 technology has become one of the most extensively used methods for introducing precise modifications into plant genomes. Although numerous tools exist to help design effective guide RNAs, *in vivo* validation remains the most reliable approach for confirming guide efficiency. *In vivo* systems enable accurate assessment of off-target effects, which is particularly important for long-lived plant species where backcrossing to eliminate unintended mutations may take several years. Given the substantial time and resource investment required for plant genome editing, a robust strategy for preselecting effective guides is essential.

In this study, we evaluated two *in vivo* approaches for validating CRISPR guides intended for genome editing in durum wheat. The selected guides were previously shown to induce editing events in the *CM3* and *CM16* genes, which encode amylase-trypsin inhibitor (ATI) proteins.

i. First method: Both the guides and their corresponding targets were included in a single plasmid construct, which was used to transform tomato via *Agrobacterium rhizogenes* to induce the formation of hairy roots.
ii. Second method: Guides were incorporated into a plasmid containing all components necessary for genome editing and delivered into durum wheat protoplasts using electroporation or polyethylene glycol (PEG) treatment.

This study contributes to enhancing CRISPR-Cas9 applications in durum wheat by refining the guide RNA selection process.

118 - Screening and evaluation of the antimicrobial activity of selected essential oils and plant-derived metabolites for the formulation of sustainable antimicrobial terpene-based cocrystals

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Plants produce a wide range of metabolites with interesting applications. Among these, terpenes, the main components of essential oils (EOs), are known for their antimicrobial and insecticidal properties. Generally considered safe for humans and the environment, terpenes are promising "green pesticides" for uses like protecting archives or controllling bee pathogens. However, their high volatility poses challenges, requiring systems to modulate their release. This study aims to formulate cocrystals, crystalline structures combining a terpene and a compound (coformer) in defined ratios. Cocrystals can slow down the release of compounds, enhancing their efficacy. The goal is to develop a mixture of multiple terpene-based formulations that mimic natural EOs composition and limit pathogens resistance. Moreover, the idea is to exploit mechano-chemical methods, which ensure greater sustainability. In the first phase of our study, the activity of eight EOs was evaluated *in vitro* against selected microorganisms. The most effective EOs were analyzed through GC-MS to identify main terpene components. The compounds thymol, carvacrol, menthol, and eugenol were then selected and tested to determine their Minimum Inhibitory Concentrations (MICs) and Fractional Inhibitory Concentrations (FICs). FIC analysis assesses potential synergistic effects between terpenes or terpene and coformer, reducing formulation doses. These findings will guide the preparation and characterization of cocrystals.

Keywords
Essential oils, terpenes, cocrystals, antimicrobial activity, green solutions

128 - Neoproteins for human nutrition

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Increasing the nutritional value of seed storage proteins (SSPs) has been largely hindered by the limited understanding of how the features of the various SSP domains determine high accumulation in protein storage vacuoles (PSV) or as endoplasmic reticulum (ER)-located insoluble protein bodies (PB).

We had designed, and tested in transgenic model plants, zeolin, a fusion formed by entire phaseolin (PHSL, the main bean SSP, located in PSV) sequence and the N-terminal domain of 27kD ⍰-zein (27⍰z, a major maize PB prolamin). Zeolin forms insoluble homomeric PB very similar to natural maize heteropolymeric PB, indicating that the zein domain has a dominant effect over PHSL. Zeolin has a more balanced essential amino acid composition compared to the two original proteins. Recently, we have determined that another maize PB prolamin (16kDa ⍰-zein, 16⍰z), which evolved from 27⍰z following maize genome duplication, remains partially soluble and forms unusual structures in the ER when ectopically expressed. We now produced zeolin2 (zeo2) by fusing PHSL to the N-terminal domain of 16⍰z. We show that zeo2 is more soluble, does not form PBs, and in relevant proportion exits the ER reaching the vacuole, indicating that the 16⍰z portion fails to dominate over PHSL features. Zeo2 is also more accessible than zeolin to proteases during the early stages of seed germination. These findings extend the possibilities of creating tailor-made neoSSPs.

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152 - Linking protein-pigment interactions with spectral phenotypes: a synthetic biology approach in microalgae

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The unicellular microalga *Chlamydomonas reinhardtii* is an unvaluable model system in the study of eukaryotic photosynthesis thanks to a rich dedicated molecular toolkit. We established a synthetic biology pipeline which combines gene editing, construct assembly and mutagenesis, to achieve the inactivation of endogenous photosynthetic genes and their replacement with modified variants at varying complementation levels.

We employed this approach to investigate the roles of protein-pigment interactions in the light-harvesting complex (LHC) protein Lhcbm1, the most abundant major antenna subunit of photosystem II (PSII). We describe a Lhcbm1 gene-dosage effect on the amplitude of the non-photochemical quenching (NPQ) response, highlighting the crucial contribution of this trimer subunit to the feedback de-excitation photoprotective mechanisms. Furthermore, by preventing the binding of selected chromophores via amino acid substitutions, we could reveal the putative roles of chlorophyll A2 (*a*612) and B3 (*a*614) as Lhcbm1 quenching sites and/or their involvement in the transfer of excess excitation to the interacting protein LhcsR3, the main NPQ effector in this organism.

Finally, by shortening the inter-chromophore distance of a chlorophyll dimer (A5, *a*603 - B5,*a*609) we could shift chlorophyll *a* absorption of Lhcbm1 towards lower energy wavelenghts.

Altogether, our strategy affords the systematic analysis of pigment-protein and inter-pigment interactions in the light-harvesting apparatus, to investigate basic physiological processes, and to explore new rationally designed LHC isoforms with tailored spectral properties.

154 - Insights into the Antioxidant and Antimicrobial Effects of Selected Indigenous Kenyan Medicinal Plants

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Defined by the World Health Organization (WHO) as indigenous knowledge and practices used for maintaining health and treating illnesses, traditional medicine (TM) represents a rich reservoir of ancient healing practices rooted in cultural traditions and accumulated wisdom over centuries. Five indigenous Kenyan plant species traditionally used in African TM, named Afzelia quanzensis, Azadirachta indica, Gigasiphon macrosiphon, Grewia bicolor, and Lannea schweinfurthii, represent a valuable resource in healing practices, yet their chemical composition and bioactivity remain understudied. To depict a primary bio-chemical characterization of these plants, their antioxidant and antimicrobial features have been evaluated by the use of methods validated in this context. G. bicolor, and G. macrosiphon were found to have great potential as sources of bioactive metabolites, such as chlorophyll a (1456.29 µg/ g DW; 1104.33 µg/ g DW), chlorophyll b (712.48 µg/ g DW; 443.31 µg/ g DW), and carotenoids (369.71 µg/ g DW; 300 µg/ g DW) as well as phenols (31.78 mg GAE/g DW; 27.54 GAE/g DW), and exhibiting high antioxidant activity, according to TEAC, DPPH and FRAP assays. Additionally, L. schweinfurthii and G. macrosiphon demonstrated antimicrobial activity against the Gram-negative bacteria E. coli, as well as against Gram-positive ones, S. aureus and B. subtilis.

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